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POLLINATOR SAFETY IN AGRICULTURE



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POLLINATOR SAFETY IN AGRICULTURE

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This publication provides guidance on the natural history of wild bees and their potential exposure to pesticides, as part of the GEF supported Project “Conservation and Management of Pollinators for Sustainable Agriculture, through an Ecosystem Approach” implemented in seven countries – Brazil, Ghana, India, Kenya, Nepal, Pakistan and South Africa.

The project is coordinated by the Food and Agriculture Organization of the United Nations (FAO) with implementation support from the United Nations Environment Programme (UNEP).



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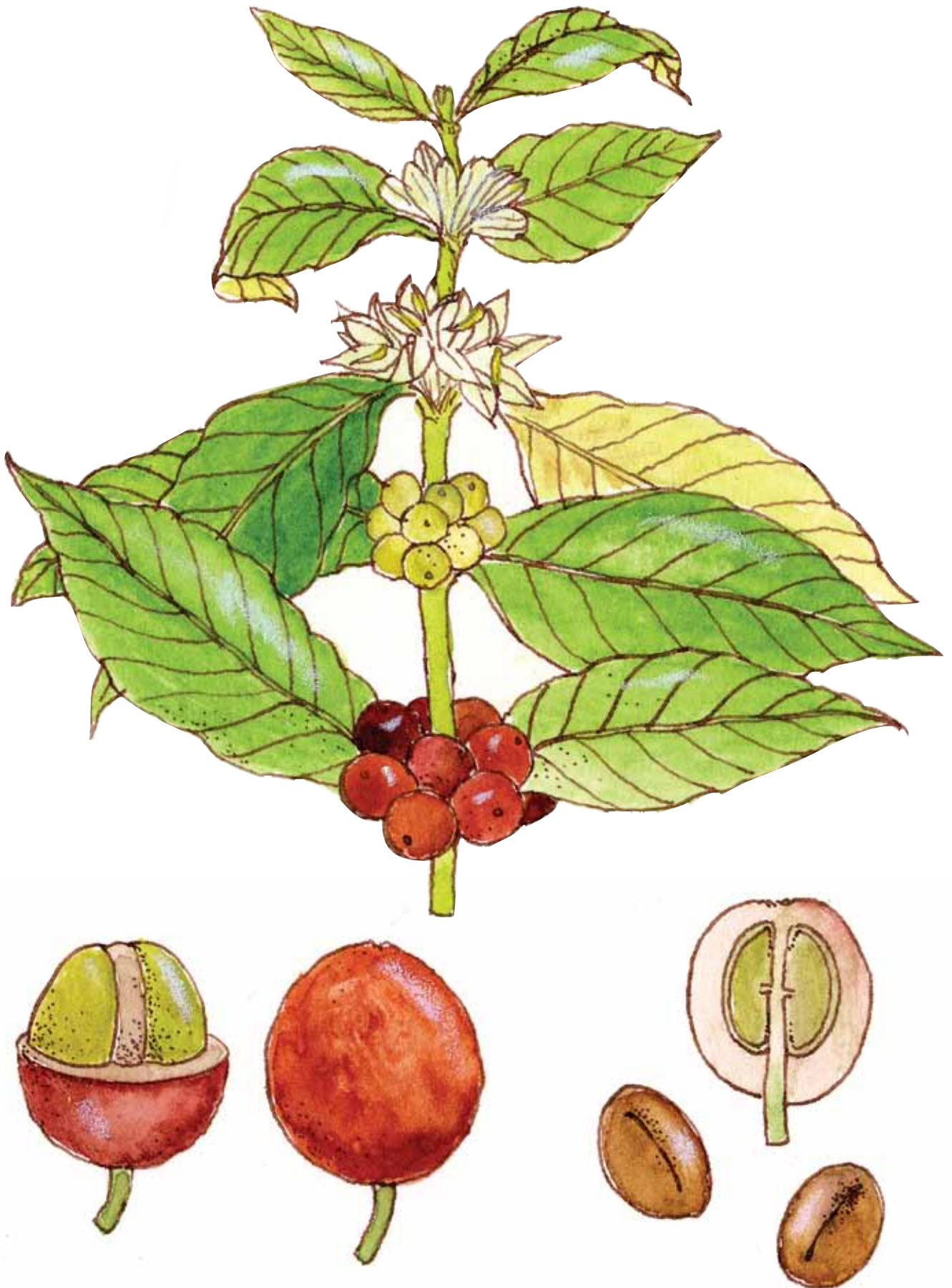
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Pollinator-dependent crop: coffee plant with flowers and berries, details of coffee berries.

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PREFACE

Pollinators are an innocent casualty of the war on insects. Noted 50 years ago by Rachael Carson¹ in her book *Silent Spring*, and continuing to arouse great concern in natural science and agriculture, the unintended biocide poisoning of wildlife persists. The insects, many of them bees, sustain agriculture and wildlands by collectively allowing crops and wild plants to reproduce. No pollinators would mean no seeds or fruit, and therefore the collapse of agriculture – and no plant reproduction in the wild means that many plants become locally extinct. The chemicals and pesticides used for seeds, plants, livestock and even pets find their way to non-target animals through the rain, air and the soil. We are at a crossroads at this moment, with new data now rapidly coming in. The well-known honey bees and bumble bees are failing to adjust to pesticides in the temperate zone. Yet we know nothing of most other pollinator groups, and terribly little about the tropical part of the world. In biologically rich and productive regions, the “free” pollinators are taken for granted, because until now, they have lived alongside the human communities that rely upon them. Is it too late to change course? What can be done about the way agricultural biocides are tested or applied? Here we examine, in detail, how pesticides and bees are intertwined, and how our knowledge can be applied toward avoiding ecological disasters that are certainly threatening to take place.

Thematic studies of stresses that affect living things compel us to wonder which stresses are normal, and which are not. The global warming driven by greenhouse gasses, the resultant melting of glaciers, lowered flow of rivers fed by melting ice in the high mountains, and a resultant rise in sea level are major concerns that affect almost all of life. Such changes are accelerating to the extent that previous shocks and struggles for the earth’s biota are small by comparison. Rainfall is intensifying where rains are normally abundant, yet droughts are

¹ Carson, R. 1962. *Silent Spring*. Houghton Mifflin, New York.



more frequent and more severe, both in the temperate zone and in the tropics. Forests and wildlands are being removed at an increasing rate, agriculture is intensified and its territory expanded, while the cities are growing. At the same time, many small-scale croplands and village domains are shrinking, leaving less of the mosaic environment in which humans and a rich biota may coexist. In the meantime, humans in all agricultural and urban areas are urged to use biocides, sometimes without sufficient protection to themselves. It may not be far-fetched to someday find much evidence of a sad truth, foreseen in Carson's *Silent Spring*, in the chapter "A Fable for Tomorrow": "No witchcraft, no enemy action had silenced the rebirth of new life in this stricken world. The people had done it themselves."

A careful look at pollinators can help us understand how they may live and carry out their vital function in our world, and how we can manage not to destroy or poison much of it ourselves. Bees and other pollinators are not a feeble or helpless group. On the contrary, they have extraordinary capacities of flight, homebuilding, and food seeking, as well as many defenses from natural enemies, both small and large. In the brain of a bee there is a map of the environment, and a sharp memory of where food and stress sources exist. The complex dynamics of many things are learned by bees. They make a living by making the right choices, permitted by gathering the correct information. Our struggle to understand and maintain our own environment in a healthy state closely matches the bee's instinctive pursuit.

The greening of pollination is our goal. That is, native or wild pollinators can be sustained, while those sought and utilized in agriculture can benefit from the same practices and insights. Our human environment will also become safer, as our crops receive the benefits that only the pollinating animals can bring them. This book, keyed to practitioners in the tropical world, testifies that we can positively alter the way food is produced by managing agriculture to avoid known exposure risks of pollinators to pesticides. Because environmental quality issues are pressing concerns for all, maintaining and protecting pollinators is, in the final analysis, the same pursuit as the conservation and green management of farming, forestry and wildlands, and of maintaining our ever-expanding garden.

In its role as coordinator and facilitator of the International Pollinators Initiative (IPI) of the United Nations Convention on Biological Diversity, FAO established a Global Action on Pollination Services for Sustainable Agriculture. Within the Global Action, and through the implementation of a GEF/UNEP-supported project on the "Conservation and Management of Pollinators for Sustainable Agriculture, through an Ecosystem Approach", FAO and its partners in seven countries — including Brazil and Kenya — have been developing tools and guidance for conserving and managing pollination services to agriculture.

A complementary initiative on “Knowledge management of pesticide risks to wild pollinators for sustainable food production of high-value crops” has been undertaken with participation of national partners in Brazil, Kenya and the Netherlands. That work has included the development of profiles for pesticide risk to wild bees in focal crops. A key element of any organism’s pesticide risk is its natural history, and the routes by which it may be exposed to pesticides in its foraging and nesting activities. In this respect, a series of presentations on the natural history of wild bee groups and pesticide exposure were solicited for a session on “Exploring pesticide effects on non-*Apis* bees” at the X International Symposium on Pollination, convened by the International Commission on Plant-Bee Relations, in Mexico, 27-30 June, 2011. The presentations have been more fully developed for the present publication, as a contribution to knowledge management of pollination services in sustainable agriculture.

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Two worker Morrison's bumble bees (Bombus morrisoni) sonicate the pollen from pored-anthers of a garden tomato.

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CHAPTER 1

AN AGROECOSYSTEM APPROACH TO PROTECTING POLLINATORS FROM PESTICIDES

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David Ward Roubik

INTRODUCTION

Food security is supported by pollinators, which make a contribution estimated at US\$ 220 billion each year (Gallai *et al.* 2009), representing 9.5 percent of the world's agricultural food production. In particular, many fruits, vegetables, oil crops, stimulant crops (coffee, tea and other beverages), along with nuts and seeds depend on animal pollination. Honey bees and bumble bees are the best known pollinators, but the wild bees – a much larger group – are essential for the pollination of many crops.

The dependence on managed pollination services for agricultural production is increasing, as agriculture intensifies. At the same time, worldwide there is evidence that insect pollinators are in decline. That decline is tentative, considering the lack of comprehensive data (LeBuhn *et al.* 2012), but it is still a pressing concern. Various causes for decline have been identified. Losses in diversity and abundance are particularly strong under intensive agricultural management (Biesmeijer *et al.* 2006; Klein *et al.* 2007; Le Féon *et al.* 2010). Also associated with agricultural intensification are habitat loss and pesticide application, both of which contribute to the loss of insects, including pollinators (Brittain *et al.* 2010; Tasei 2002; Tuell and Isaacs 2010).



To address the issue of pollinator decline, in 2000, the Fifth Conference of the Parties of the Convention on Biological Diversity (CBD) established (COP decision V/5), the International Initiative for the Conservation and Sustainable Use of Pollinators (also known as the International Pollinator Initiative – IPI). Priority actions of this initiative, outlined in the IPI Plan of Action¹, are the monitoring and assessment of actual and potential impact from agricultural technologies, including pesticides, on pollinator diversity and abundance (CBD 2002).

Historically, pesticide risk assessment for pollinators has been based on information related to only one species, the Western honey bee (*Apis mellifera*). However, there are more than 20,000 species of wild bees, and for many plants, those bees are more important pollinators than honey bees. Nonetheless, wild bees are often low in numbers - particularly under intensive agriculture, thus managed colonies of honey bees and bumble bees are used to provide pollination services, which would otherwise be lacking. The assessment of pesticide risk to pollinators other than the Western honey bee has remained in its infancy (Fischer and Moriarty 2011). The information brought together in this publication is intended to help organize and apply existing knowledge on pesticide risk to the many and diverse non-*Apis* bees, while the gaps in knowledge are further reduced.

THE JUDICIOUS USE OF PESTICIDES

In most instances the first step, when faced with insect pests that might seem to require control by pesticides, is to take a wider view of the problem. If pesticide exposure affects pollinators, it is critically important to assess agroecosystem management practices.

A wide range of ecosystem functions are governed by predators, parasites and pollinators, their competitors, and even by pests of agricultural and silvicultural crops (FAO 2011). Although plant pests are often thought of as destructive, they occur naturally and only rise to outbreak levels when their control by predators or parasites is reduced.

Integrated Pest Management (IPM) begins with an assessment of the local agroecosystem, to understand possible causes for disruption of natural balances between insect pests and their mortality factors. For example, Farmer Field Schools (FFS) have been successful in Asia and Africa, to help farmers address crop production issues. Farmers undertake an “agroecosystem analysis” as a core exercise. That exercise encourages individuals to evaluate field conditions and crop growing needs, and to understand the population dynamics of pests and their natural enemies. In such an approach, pesticides are to be used only as a last resort. However, the monitoring of pests or their

1 <http://www.cbd.int/decision/cop/?id=7179>

natural enemies—to establish whether there is actual need for pesticides—depends on detailed knowledge and correct field identification of insect species. Therefore, while following a plan of minimising pesticide costs with such applied knowledge is ideal, it is often beyond the means of the individual practitioner. In addition, even though specialized equipment, such as a hooded pesticide sprayer, is available to greatly reduce drift and exposure in pesticide application, such machinery is costly. The individual grower or farm manager often faces such technical problems, for which the solutions depend both on innovation to achieve desired economic goals, and avoiding destructive practices considering pollinators. There are several means to potentially consistently serve both goals, and to maintain necessary safety standards.

A cautionary tale with respect to pollinators and pesticides is presented in Chapter 2. While not a story about wild bees, it describes how even beneficial insects can be mistaken for pests. The example underscores the importance of carefully evaluating the roles of particular organisms in agroecosystems. In many instances, pest outbreaks may be caused by the misuse of pesticides, which kill natural enemies and thus allow a pest outbreak. Similarly, pesticide misuse may kill pollinators. The results are not immediately obvious and may only be perceived later, indirectly, through reduced crop yields.

In recent years the attempt to optimize pesticide application in crops has shifted towards use of pesticide-coated seed. There are flaws in the application methods that have released pesticide from the seeding machine at the time of planting, contributing to bee mortality or affecting bee behavior. In Europe, evidence of bee deaths or affected behaviour, possibly from such practices, has led to temporary suspension in the use of some chemical pesticide products for maize. This was deemed necessary to allow time to assess the impact on honey bee health.

A typical problem of agricultural intensification is illustrated by the aforementioned pesticide-coated seed case, which relates to decision-making and pesticide use. Pesticides applied with seed treatments are frequently used in mono-cropping, with the aim of controlling insect populations that proliferate to pest status when the same crop is produced for several years. From an ecosystem perspective, to achieve sustainability the appropriate management response should draw on integrated approaches including practices such as crop rotation, and not solely the use of pesticides. The practices that enhance sustainable crop production and pollinator abundance may not be limited to reducing pesticide exposure. They may be seen as larger issues of appropriate decision-making in cropping system management.

When it is recognized that insect populations, including pests, are naturally present in agricultural fields, a few regular preventive practices—such as crop monitoring, rotation and spot control measures—will usually keep pests at low levels. The eradication of an insect pest is



rarely feasible, or desirable. Pest eradication would eliminate the food supply of the pest's natural enemies, removing a key element in system regulation. The objective of sound pest control is to manage insect pest populations to the point where natural predation operates effectively and crop losses to pests are kept at an acceptable minimum. Where pesticide applications are considered necessary, however, an assessment of their possible impacts on all beneficial insects, including pollinators, is required.

A PROPOSED STRUCTURE FOR RISK ASSESSMENT ON NON-*APIS* BEES

To carry out an appropriate risk assessment of pesticides to wild bees, or to non-*Apis* managed bees, information is needed on: (1) pesticide toxicity; (2) the probability of exposure to the pesticide; and (3) the impact of natural history and population dynamics on bee abundance or behavior in response to stress. Such risk factors can sometimes be ranked, or a single factor recognized as of primary importance. In some cases, a clear causal relationship can be assumed between the factor and an increase or a reduction of risk; in other cases this relationship is less clear and requires more detailed study. Annex 1 provides a list of factors that potentially influence the risk of pesticides to bees, classified according to the three categories listed above.

(1) Pesticide toxicity: The relative toxicity of different pesticides to pollinators is of fundamental importance in risk assessment. Although most studies on pesticide toxicity have focused on honey bees, recent years have witnessed increasing efforts to carry out similar or comparative studies with non-*Apis* pollinators. Previous research has indicated that toxicity may vary across different bee groups. For example, Torchio (1973) studied the comparative susceptibility of the honey bee, an halictid bee and a megachilid bee in the United States; Scott-Dupree *et al.* (2009) compared bumble bees and two species of megachilid bees visiting canola in Canada, and Valdovinos-Núñez *et al.* (2009) assessed pesticide toxicity for different species of stingless bees in Mexico. These and the majority of comparative studies focus on pollinators of a crop in one agroecosystem or geographic location. An initiative supported by the Netherlands permitted a comparative study of wild bee susceptibility to pesticides within a wider range. It includes native bees from Europe, Brazil and Kenya (Roessink *et al.* 2011). Identical tests were set up with *Bombus terrestris*, *Apis mellifera mellifera*, *A. m. scutellata* (Africanized), *A. m. scutellata* (native African), and two stingless bees, *Scaptotrigona postica* and *Meliponula ferruginea*. Preliminary results using two insecticides (deltamethrin and dimethoate) show that the European honey bee was not the most sensitive species tested. Sensitivity of *A. m. mellifera* was less by factors of 15 and 2.5 for deltamethrin and dimethoate, respectively. The basic toxicity trials for honey bees are obtained from oral (feeding) and contact laboratory studies. Bee brood

toxicity tests and studies with pesticide residues may also be conducted if the mode of action of the pesticide, or route of exposure to the bee, warrant such an approach. Furthermore, semi-field and field trials are attempted if the laboratory data are equivocal (EPP0 2010a). The laboratory studies, in particular, tend to focus on mortality and do not assess sub-lethal effects such as behavioural impairment or abnormal development. Whether or not to incorporate such sublethal effects in honey bee toxicity testing has been recently considered (Thompson and Maus 2007). However, because even the lethal dose trials are largely absent for wild bees (with exceptions among bumble bees), adequate information on sublethal effects of pesticides is still unavailable.

(2) Probability of exposure: The probability and degree of exposure to pesticides depends on many factors that can be categorized according to cropping factors, pesticide application and chemical properties, and also varying with bee biology.

Cropping factors that may influence bee exposure include timing of sowing or planting (and subsequent flowering), bee attraction to the crop (and to other flowering plants in the crop area) and the suitability of cropping environs, like field margins or neighbouring wildlands, for bee nesting.

The pesticide application method, including its rate, timing, and frequency, as well as pesticide properties such as the type of chemical formulation, the degradation half-life and possibility of translocation in the plant, also influence exposure.

Risk is determined by when and where bees forage, live and reproduce. Bee phenology (their seasonal development, adult emergence and activity patterns) and also their behavior will determine exposure to pesticides when they are flying and visiting flowers, or nesting in a wide variety of localities. With respect to space, for example, the exposure of bees that nest in the fields where pesticides are being applied is likely to be much higher than those nesting farther away. Bees that have large foraging ranges are likely to diminish their exposure by visiting a larger diversity of crops and flowers, some differing in pesticide load. With respect to active periods and tempo, some bees may emerge and complete their life cycle almost entirely within the blooming period of a crop, making them completely exposed to all pesticide applications. Other bees may have more prolonged life cycles with reproduction taking place before crop bloom. Such bees may experience less risk of pesticide exposure.

A comparison of honey bees to most wild bees illustrates how feeding behaviour may cumulatively impact exposure. All bees, particularly adults, consume nectar as their source of carbohydrates, and all (except for four *Trigona* in the Neotropics) developing bees - the larvae - consume pollen as a protein source. An adult bee consumes relatively little pollen during its life. Honey bees collect pollen and nectar from a wide range of plants, whereas

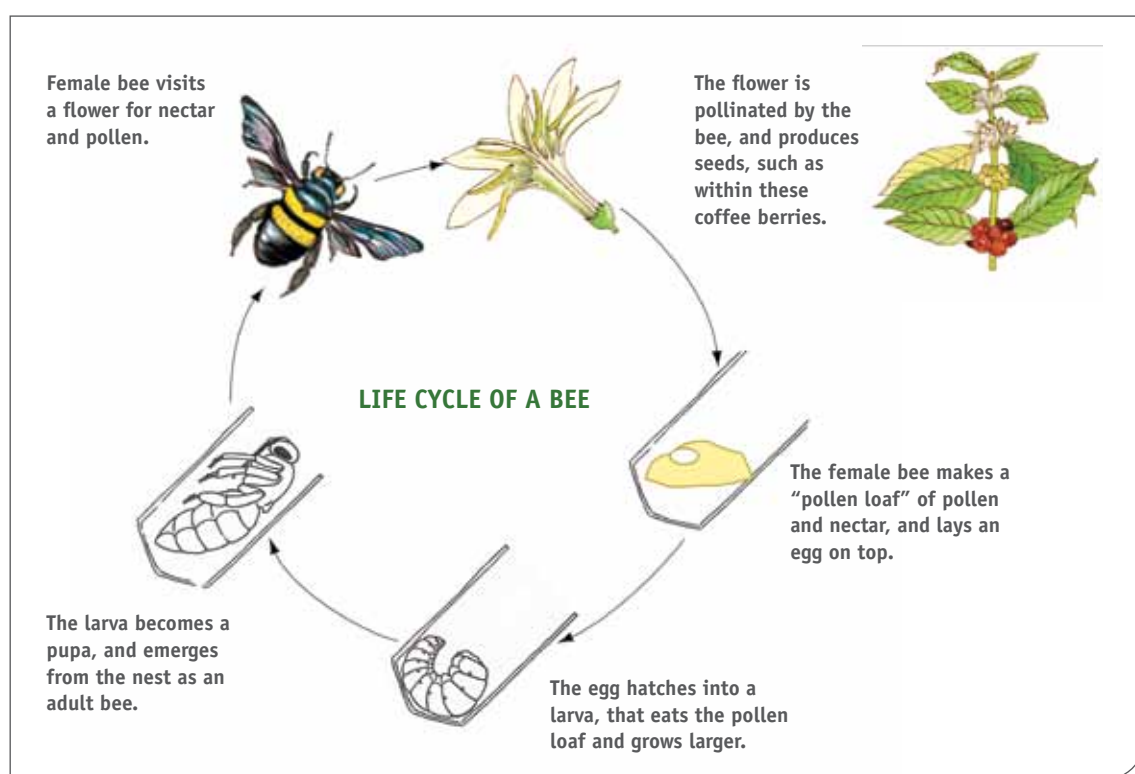


some wild bee species forage over much smaller areas, may nest in or close to fields, and some individuals may gather resources largely from a single crop, making them more vulnerable to a pesticide treatment on that crop. Many bees collect other material, including soil, wood, mud, resin, leaves or leaf hairs used to build nests, and they may also collect water, sap from wounded plants or sap-feeding insects, or floral oils, used to feed larvae. When any such materials are contaminated with pesticide there is an additional risk factor.

Bee exposure to pesticides is not, however, limited solely to the field where those products may be applied. Bees have a multi-stage life cycle (Figure 1.1). For most bees (those not parasitic on other bee species) the female provides food to the immature brood in a nest, with a mixture of nectar, pollen and sometimes oil. Honey bees 'process' their pollen and nectar, and the youngest larvae are

Figure 1.1

LIFE CYCLE OF A FEMALE CARPENTER BEE



Bees have a multi-stage life cycle, from egg, to larva, to pupa, and finally the adult bee. For simplicity, the mating behavior needed in any bee life cycle is omitted here, although it should be noted that nesting does not take place before mating. Male bees, furthermore, are usually not living in a bee nest, but in the wild, frequently exposed to any risk factor throughout their lives.

largely fed royal jelly, produced in the glands of worker bees. The glandular processing may degrade toxins, whereas most wild bee larvae are fed comparatively 'unprocessed' nectar and pollen.

Bee exposure to pesticides can be avoided if sprays are timed for periods when bees are not active, such as at night. However, the timing of bee activity is actually quite diverse, both over a year and during a day. While the colonies of social bees such as honey bees and stingless bees may exist for many years, most other bees live for only one season. This may be a single season in the temperate zone, or multiple cycles in the tropics. Wild bees also often forage earlier or later in the day than honey bees, or when temperatures are lower.

Bee behavior and natural history are certainly key factors in risk assessment and risk mitigation for wild bees. Even in cases where it is possible to predict relative toxicity (on the basis that larger bees may be less susceptible – although this is not consistently true) the behavior of different wild bee species in the field differs considerably from honey bees. The mitigation measures that protect honey bees may therefore not be as effective in protecting other bees. This was shown in bumble bees by Thompson (2001). Recent research and pollinator conservation programs aimed solely at reducing insecticide use have had varying success, related to the biology of the target bee species (Tuell and Isaacs 2010). By modifying practices according to what is known of the natural history of key pollinators, it may be possible to diminish their exposure.

(3) Impact of natural history and population dynamics on bee abundance or behavior in response to stress: These species-specific characteristics may determine the cumulative lethal or sublethal pesticide effects on long-term survival of the population.

Honey bees and other highly social bees have colonies with thousands of individuals, whereas the large majority of wild bees are solitary; the female bee mates, provisions nests, forages for resources and lays eggs. A solitary female bee, succumbing to pesticide exposure, will not be able to produce further offspring, whereas a bee colony is capable of continuing to produce more bees, despite the loss of individual workers, or even the queen.

Fecundity of wild bees is far more limited than that of honey bees. The queen of a honey bee that has mated with 10-30 males has a lifetime supply of sperm to fertilize eggs during her life of at least a year. The queen of a stingless bee, however, mates with a single male. The range for less social or solitary bees may be seen, for example, among the small colonies or groups of African carpenter bees (*Xylocopa* spp.) which only produce 1 to 5 offspring per generation, and European mason bees (*Osmia rufa*) that produce up to 20 offspring. This contrasts with up to several tens of thousands of offspring per queen for *Apis mellifera*. Fewer offspring mean that after bee mortality caused by pesticide, recovery will be slower.



CURRENT STATE OF KNOWLEDGE AND NEEDS ON PESTICIDE RISKS TO WILD POLLINATORS

Regulatory procedures for pollinator risk assessment have hitherto focused entirely on Western honey bees, in Europe (EPP0 2010b), the USA (EPA 2011) and Australia (EPHC 2009). In most cases the methods cannot easily be adapted to wild bees. Why? Because wild bees are a far more diverse group than we are often led to believe. Most pollinating bees are solitary and seasonal, not social and perennial. Moreover, they are not directly bred or looked after by humans (although the agricultural and other practices imposed on the environment, by humans, strongly influence their success or failure as components in a major ecological event—pollination—which produces our food). That interaction can sustain human food ecology, or it can fail to provide adequate pollination services. The latter may lead to the collapse of cultivation of pollinator-dependent crops in today's agricultural systems. Recent studies have made some impressive conclusions on the risks to pollinators of certain classes of pesticides, developed to eliminate direct risk to humans (Bommarco *et al.* 2012; Whitehorn *et al.* 2012). It is now appreciated that while chemical research and development have been beneficial in the short term, the impact of these chemicals, such as the neonicotinoids, has yet to be screened in a way that shows they are not a threat to pollinators. There is no reason to suspect that such discoveries are unique, or restricted to the honey bees or bumble bees, which have been studied in some detail.

In response to growing international concern over a decline in diverse bee species, initiatives are underway to refine and elaborate pesticide risk assessment practices, and to include wild bees. The Organisation for Economic Co-operation and Development (OECD) carried out a survey of “Pollinator Testing, Research, Mitigation and Information Management” in 2009. Its objective was to gather information related to pollinator decline, with a specific focus on possible relationships with pesticides. The survey, with responses from 17 OECD member countries, indicated much concern about bee and other pollinator declines. It also revealed commitment on the part of almost half the countries to expand toxicity tests and make the risk assessment for pollinators more effective (OECD 2010).

In January of 2011, the Society of Environmental Toxicology and Chemistry (SETAC) held a workshop to explore the state of science concerning pesticide risk assessment for pollinators (Fischer and Moriarty 2011). One of the workshop goals was to explore the applicability of testing protocols used for *Apis* to measure effects of pesticides and pesticide risk on native (non-*Apis*) bee species. The workshop report noted that the biology and ecology of non-*Apis* bees differs from honey bees in a number of aspects that may be important in risk assessment

for pesticides. While the workshop proposes pesticide risk assessment schemes also for wild bees, these have yet to take into account the specific toxicity, exposure and population dynamics factors mentioned above.

It is apparent that breakthroughs in pesticide and risk assessment for honey bees are on the right track (e.g. Gill *et al.* 2012; Mao *et al.* 2013). The European Food Safety Authority (EFSA) is currently in the process of developing guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). The draft risk assessment guidelines should explicitly take into account non-*Apis* bee species (EFSA, unpublished).

Recent work under the European Union (EU) ALARM project has also contributed to comparative risk assessment for wild bees (Barmaz *et al.* 2010), but needs further development aimed at regulatory decision making. A recent study of factors that may determine the risk of pesticides to wild bees, on three continents, has shown that large data gaps still exist on biology, life-history and population dynamics of bees among various pollinated crops (Van der Valk *et al.* 2013). This greatly complicates proper risk assessment, i.e. making reliable inferences about the magnitude and duration of adverse pesticide effects on wild bees. As an alternative, the authors of the study propose qualitative "risk profiles", to indicate the likelihood of pesticide impact on bees and pollinators in general in specific cropping systems (Annex 2). The data collected through such risk-profiling should increase the knowledge of pesticide risks under varied circumstances, and ultimately contribute to the development of more specific risk assessment procedures.

CONCLUSIONS

While the information in this publication is insufficient to instruct practitioners on pest control using the highest possible personal and environmental safety standards, while minimizing their cost, the readers' attention is called to some of the most promising ways available to accomplish these goals. One of them is using biological control, including parasites, predators and pathogens such as bacteria, which are not harmful to humans or non-target wildlife. Similarly, the placement of "capture and kill" and "bait traps", using either chemical pheromone mimics or other means of removing insects from a crop area, are cost-effective and increasingly available for a specific insect. In contrast, the conventional application of agricultural chemicals may expose people and the environment to toxic substances. Thus, in many cases, it becomes a "win-win" solution to pursue alternative pest control strategies, with benefits for human and pollinator health as well as for minimizing costs of inputs. When the fundamental conclusion made from the evaluation procedures detailed in Annexes 1 and 2 makes it clear that pollinators will be adversely affected, it is strongly suggested that other control methods are employed.



Risk assessment approaches and procedures to address non-*Apis* bees are still in their infancy, and the life histories of honey bees and bumble bees do not adequately encompass other bee species. Because pesticide risk assessment should be relatively simple and practical to gain wide application, consideration of different bee life histories that influence pesticide exposure is of fundamental importance.

Pesticide exposure routes vary, according to the biology of bees that pollinate different crops. Together with specialists, information was assembled, in as much quantitative detail as possible, on the aspects of wild bee biology relevant to pesticide exposure risk. In the rest of this publication there are chapters on: pesticide exposure among wild bees in Brazil (including highly social stingless bees (the Meliponini, Chapter 3); the natural history and pesticide exposure for primitively social bumble bees (*Bombus*, Chapter 4); the solitary leafcutter and mason bees (Megachilidae, Chapter 5); small solitary and social bees (Halictidae, Chapter 6); and two large bees (*Xylocopa* and *Amegilla*, Chapter 7). Specific recommendations for incorporating their natural history into risk assessments are given. This information also applies to pesticide risk assessment for pollinators other than bees. We hope that it may guide both risk managers and pesticide users.



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Top: apple blossoms in Kullu Valley, Himachal Pradesh; below: Eastern honey bee (Apis cerana) on apple blossoms.



CHAPTER 2

WILD POLLINATORS AND PESTICIDES ON APPLES IN HIMACHAL PRADESH, INDIA: COMMUNITY LEARNING AND INNOVATION

Jitendar Kumar Gupta

INTRODUCTION

Himachal Pradesh is known as the “Apple State” of India. More than 90,000 hectares of land are committed to apples (*Malus domestica* Borkh.), which bring in an annual contribution to the State economy that is estimated at US \$1.7 billion. Most of this income (\$1.5 billion) is related to revenues during the six-month growing season. Thousands of people, not only in Himachal Pradesh but also in Delhi (host to Asia’s largest fruit market), benefit directly or indirectly from the State’s apple growing industry.

PERCEPTION OF WILD POLLINATORS

In the early 1990s, the appearance of a “yellow fly” during the apple blooming months of March to April caused considerable alarm among apple growers. The “yellow fly”, as they referred to it, had been observed on apple blooms in large numbers. Some apple orchardists began using insecticide to control the fly while others thought to enquire, from scientists, as to which insecticide should be applied.

Apiculture scientists were already working with apple farmers, advocating the use of honey bee colonies to increase apple productivity. This involved setting up demonstration trials on farms, which farmers had not been actively managing. The event of the yellow fly was to prove a critical opportunity allowing farmers to see for themselves the role of pollination in their apple orchards.



Upon receiving reports of the infestation, the Head of the Department of Entomology and Apiculture of the Y.S. Parmar University of Horticulture and Forestry (Nauni, Solan, Himachal Pradesh) appointed a team of three scientists: one from the biological control section, another from toxicology and Dr Gupta [the author of this chapter], representing the apiculture section.

FINDINGS

The team went out on field missions to three apple growing areas covering three different districts: Kotgarh in Shimla district, Churag in Mandi district and Kullu in Kullu district. In each of these areas, farmers met with the scientific team, describing the infestation of the yellow fly as that of a pest.

The scientific team was able to identify this insect as in fact a syrphid fly, *Episyrphus*, whose population was unusually high during that year. They explained to the apple farmers that this fly is actually a very useful pollinator, which varies in its abundance from year to year. The adult flies eat pollen and, in so doing, transfer pollen between flowers, thus bringing about pollination. As such, large population years are a bonus, and not a threat, to apple production.

As it is, during the apple bloom period early in the year, weather conditions are unstable. When low temperature conditions occur, honey bees may not visit apple trees in bloom, but these flies remain in abundance at such times.

Other benefits of *Episyrphus* and other syrphid flies are in their aphid-eating larvae, which may be important to control aphids on fruit trees (Sharma 2001). The adults may also pollinate other crops, such as cauliflower for seed production (Kapatia 1987). Apple orchardists were advised not to spray insecticide on a blooming apple crop as a measure against yellow fly.

Figure 2.1a

***EPISYRPHUS* ON APPLE BLOSSOM**



Figure 2.1b

A PINNED SPECIMEN



Figure 2.2a and b

A TRAINING PARTICIPANT PRESENTS ORCHARD DESIGN FOR IMPROVED POLLINATION BY SYRPHID FLIES**LEARNING AND INNOVATION**

Some growers heeded the scientific team's advice while others remained doubtful and applied insecticide to kill syrphid flies. The result was that those who did not apply insecticide had good harvests and those who applied insecticide on the blooming crop had poor crop yields. The latter was the result of both lower honey bee numbers, due to pesticide application, and cold weather conditions in which honey bees were not as effective in pollination. On the other hand, for those who had favourable results, the syrphid flies worked under even adverse weather conditions, and their large population compensated to some extent for the pollination otherwise performed by bees. These comparative results went a long way in convincing farmers that the fly is a useful pollinator.

However, the Department of Entomology and Apiculture still receives complaints and requests for advice on controlling syrphid flies. With this in mind, it was decided to organize a trainers' workshop held in April 2011¹, and handouts were printed with photographs of pollinators, including syrphid flies.

¹ The workshop was part of a UNEP/GEF/FAO Project, "Conservation and Management of Pollinators for Sustainable Agriculture, through an Ecosystem Approach" with the G.B. Pant Institute of Himalayan Environment and Development as the project national executing agency in India.



One workshop participant, Mr Vijay Singh, convinced of the value of syrphid flies as alternate pollinators of apples, studied the advice given on placement of 'polliniser' trees (those that provide pollen for outcrossing pollination) in an orchard, and came up with his own design. Since syrphid flies do not fly long distances, he developed a design that minimizes the space between polliniser branches and production trees.

During interactive sessions with farmers and trainers, it came out that some orchardists were resorting to spraying methyl parathion on apple blooms. This spraying is carried out not to control any pest, but rather from a mistaken notion that the pesticide application generates heat, which provides warmth to the bloom during adverse weather conditions and helps in pollination. Although the trainers sought to correct this idea, and explained there is no scientific basis for this view, many still insisted. The trainers therefore encouraged the farmers to test methyl parathion on some trees, while leaving a control in the same orchard and observing for themselves the difference, if any. On the basis of this on-farm experiment, farmers were persuaded that, in fact, treatment with methyl parathion is not effective in any way for improving pollination and yield.

CONCLUSIONS

In principle, farmers in Himachal Pradesh have learned not to apply insecticides on apples at the time of bloom. However, questions continue about wild pollinators and the appropriate use of pesticides, with respect to pollination. The positive value of farmer training, dialogue and on-farm experiments on these issues remains very clear.



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*Inside of a nest, within a wooden hive, of the stingless bee *Meliponula ferruginea* in Kakamega forest, Kenya, and detail (below), each showing complex nest structure.*



CHAPTER 3

PESTICIDE EXPOSURE ROUTES FOR BRAZILIAN WILD BEES

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INTRODUCTION

In the diverse ecosystems of the Neotropics, the number of bee species is estimated at 3 000, including both social and solitary bees (Silveira *et al.* 2002a). In Brazil alone, there are at least 1 678 bee species (Moure *et al.* 2008). According to Michener (2007), these are distributed among five families of the superfamily Apoidea as follows: Andrenidae (82), Apidae (913), Colletidae (104), Halictidae (251) and Megachilidae (328). Brazil is an example of how far research has come in part of the Neotropics, and how much further it needs to advance.

Naturalists and taxonomists like Cockerell, Ducke and Friese, at the beginning of the 20th century, carried out early studies on Brazilian bees. From a hundred years of extensive bee-related scientific research in Brazil there are bee species lists, information on bee abundance, and details on seasonal and daily activities (Pinheiro-Machado *et al.* 2002). An extensive catalogue of Neotropical bees (Moure, Urban and Melo 2008) and a world list of genera and species are available through the websites of Discover Life (www.discoverlife.org). However, according to Freitas *et al.* (2009), there is still limited understanding on the diversity, taxonomy, distribution and dynamics of bees in tropical America. The lack of a standardized methodology, the lack of synthetic studies that summarize research, and inadequate facilities for correct identification of bees are cited as reasons for which the apifauna in Brazil could be better studied (Silveira *et al.* 2002b).



In the Neotropics, there are many threats to native bees, which are mostly related to human activities that result in habitat alteration, as well as honey hunting, invasive species (many plants and even some bees) and intensive use of pesticides (Freitas *et al.* 2009). In tropical environments, most colonial or eusocial bees forage for resources throughout the day (from around 6 a.m. to 6 p.m.), with peak pollen collection in the morning and peak nectar collection later in the day (Roubik 1989). The worker bees of such colonies may be at risk of pesticide exposure for a period of approximately 12 hours each day. Due to the tropical climate of Brazil, foraging occurs throughout the year, with a decrease in intensity in the subtropical states of the southeast and south between the months of April to September.

Brazilian law requires that a certain portion of each property or settlement must retain an area for biodiversity conservation and the protection of nature. These small forest fragments within farms are called “Reserva Legal”. Hives are often placed within these fragments (or bees may nest in the trees) putting them at the additional risk of exposure from the drift of the applied products such as biocides which may reach the forest and, consequently, the hives or nests.

Pollination initiatives like the International Pollinators Initiative (IPI) and the Brazilian Pollinators Initiative are important instruments with which to involve government, general public and researchers in a coordinated effort to inform and contribute to reducing the threats to bees in Latin America (Freitas *et al.* 2009). In 2008, Brazil became the world’s largest user of pesticides (insecticides, herbicides, fungicides, etc.) (ANDEF 2009), underlining the urgency of developing further research to study their effect on bees. By assessing both direct (survival and reproduction) and indirect (physiological, behavioral, morphological) ecotoxicological effects (e.g. Lima *et al.* 2012), new public policies and rational management plans, with a goal of protecting pollinators from toxic chemicals, can be developed.

BEE NATURAL HISTORY IN RELATION TO FORAGING

The emergence and proliferation of bees occurred in close relation with the appearance of angiosperms. The relationship between floral visitors and angiosperms is based on an exchange of rewards, where pollen and nectar are the main resources offered by the flowers. Pollen is the food essential in the life of bees as the source of protein for the larvae and young workers, while also providing lipids, vitamins and minerals (Oliveira 2009) although it might be noted that even in this, there is considerable diversity; for example there are tropical *Trigona* that do not use pollen, the obligate necrophages (Roubik 1989).

Individual bees are exposed to pesticides primarily as they forage in the field collecting pollen and nectar. It is of course female bees that collect pollen, and this sex is also the one

Figure 3.1

STINGLESS BEE REMOVING POLLEN FROM A TUBULAR ANTHOR, BORNEO

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that mixes water with soil or forages other material, in order to build the nest and form the brood cells in which the foraged pollen and nectar are placed. Such foragers may be in contact with toxic substances at the time of application, or ingest contaminated nectar or pollen. Most cases of contamination in colonies, such as those formed by honey bees, stingless bees, some halictid bees and also bumble bees, occur when pesticides are applied to flowering crops and other fields within the agroecosystem (Warhurst and Goebel 1995). Although male bees do not forage for pollen, and are not exposed to as many sources of contamination as females, they often do not live in a nest and are exposed at night or during inactive periods to any application of insecticide that arrives on foliage (Roubik 1989, 2012).

To assess the amount of possible exposure during foraging, it is necessary to consider all the circumstances that affect foraging activity, including meteorological conditions, temperature, relative humidity, distance from the source of food and bee flight periodicity and timing. In order to understand the real consequences of pesticide exposure through larval feeding, it is important to learn more about the natural history and ecology of many different bees. From data obtained for bees in field conditions, it was noted that most flight activity occurs in the morning (Table 3.1).



Table 3.1

PUBLISHED DATA ON FORAGING BEHAVIOR OF BRAZILIAN WILD BEES

	T°C FOR BEGINNING OF FLIGHT ACTIVITY	OPTIMAL T°C FOR FLIGHT ACTIVITY	AVG. RELATIVE HUMIDITY FOR FLIGHT ACTIVITY (%)	HOURS OF FLIGHT ACTIVITY (AVERAGE)	DISTANCE (METERS)	REFERENCE
APIDAE						
<i>Friesella schrottkyi</i>	19.8	-	-	-	-	Teixeira and Campos (2005)
<i>Frieseomelitta varia</i>	-	19.8	-	08:59-11:28	-	Teixeira and Campos (2005)
<i>Melipona asilvai</i>	-	21-27.4	60.6-84.5	1-2	-	Souza <i>et al.</i> (2006)
<i>Melipona bicolor bicolor</i>	11	11-18	80-89	-	-	Hilário <i>et al.</i> (2001)
<i>Melipona crinita</i>	22	24-26	-	-	-	Cortopassilaurino (2004)
<i>Melipona eburnea</i>	-	24-26	-	-	-	Cortopassilaurino (2004)
<i>Melipona flavolineata</i>	-	24-26	-	-	-	Cortopassilaurino (2004)
<i>Melipona fuliginosa</i>	-	24-26	-	-	2000	Wille (1983)
<i>Melipona marginata marginata</i>	14, 16-17	19-30	40-70	11-13	800	Kleinert-Giovanni and Imperatriz-Fonseca (1986); Wille (1983)
<i>Melipona marginata obscurior</i>	17-18	21-28	40-20	11-13	-	Kleinert-Giovanni and Imperatriz-Fonseca (1986)
<i>Melipona quadrfasciata quadrfasciata</i>	13	14-16	80-90	8-9	2000	Guibu <i>et al.</i> (1988); Kerr (1987)
<i>Melipona rufiventris</i>	16-24	-	-	6	-	Fidalgo and Kleinert (2007)
<i>Nannotrigona testaceicornis</i>	-	18.3	-	08:49-09:39	600-900	Teixeira and Campos (2005); Van Nieuwstadt and Ruano (1996)
<i>Paratrigona subnuda</i>	-	24-25	40-60	-	-	Mouga (1984)
<i>Plebeia droryana</i>	-	19.0	-	09:04-10:45	540	Teixeira and Campos (2005); Kerr (1987)
<i>Plebeia emerina</i>	16-22	21-27	40-70	13-14:30	±300	Kleinert-Giovannini (1982)
<i>Plebeia lucii</i>	21.8	-	-	-	-	Teixeira and Campos (2005)
<i>Plebeia pugnax</i>	-	22-34	30-100	-	±300	Hilário <i>et al.</i> (2001)
<i>Plebeia remota</i>	16-18	22-29	60-84	11-15	±300	Imperatriz-Fonseca <i>et al.</i> (1985)
<i>Scaptotrigona xanthotricha</i>	-	16.5	-	08:07-10:20	-	Teixeira and Campos (2005)
<i>Schwarziana quadripunctata quadripunctata</i>	14	21-26	60-99	8-13	-	Imperatriz-Fonseca and Darakjian (1994)
<i>Tetragona clavipes</i>	22-35	25-31	65	6:00-14:00	-	Rodrigues <i>et al.</i> (2007)
<i>Tetragonisca angustula</i>	17	18-23	-	8:00-9:00	600-900	Iwama (1977); Van Nieuwstadt and Ruano (1996)
<i>Tetragonisca angustula angustula</i>	17-24	20-30	30-70	11-13	-	Iwana (1977)
<i>Trigona hyalinata</i>	22	22-26	-	9-17	-	Iwana (1977)

follows on the next page →

	T°C FOR BEGINNING OF FLIGHT ACTIVITY	OPTIMAL T°C FOR FLIGHT ACTIVITY	AVG. RELATIVE HUMIDITY FOR FLIGHT ACTIVITY	HOURS OF FLIGHT ACTIVITY (AVERAGE)	DISTANCE (METERS)	REFERENCE
BOMBINI						
<i>Bombus pullatus</i>	-	-	-	7-10	-	Cameron <i>et al.</i> (1999)
<i>Bombus terrestris</i>	-	20.51	64.09		663	
<i>Bombus transversalis</i>	-	-	-	7:45-9:45	-	Cameron <i>et al.</i> (1999)
EUGLOSSINI						
<i>Eulaema bombiformis</i>	-	23-27	58-90	5:15-9:00	-	Melo <i>et al.</i> (2009)
<i>Eulaema cingulata</i>	-	24-28	58-90	5:42-8:00	-	Melo <i>et al.</i> (2009)
<i>Eulaema flavescens</i>	-	23-27	72-90	5:04-7:16	-	Melo <i>et al.</i> (2009)
<i>Eulaema nigrita</i>	-	23-26	60-90	5:20-7:45	-	Melo <i>et al.</i> (2009)
MEGACHILIDAE						
<i>Osmia cornuta</i>	-	9-12	-	7:40-6:30	-	Vicens and Bosch (2000)
<i>Megachile minutissima</i>	-	20-25	-	9	-	Shebl (2008)
ANDRENIDAE						
<i>Andrena crataegi</i>	20	15.2-24	-	7:34-19:05	-	Osgood (1989)

Few studies describe all of the localities bees visit when collecting food, because bee individual micro-transmitters for telemetry have been used only recently to track individuals (Wikelski *et al.* 2011). Most data in the Brazilian literature refer to Africanized *Apis mellifera*. However, such studies cannot be used as a basis for observations of wild bees, because, unlike *A. mellifera* that feed their larvae throughout the larval period, most other bees lay an egg and seal the cell, having no further direct contact with their developing offspring. Other highly eusocial bees, Meliponini, which store honey and pollen in the nest, are able to use some of the same means as honey bees to process and potentially detoxify their food. The Meliponini use a wide variety of materials, gathered from different parts of the environment, to build their nests. Because of their foraging at many different places within the forest and in human-made habitats, there may be more possible routes for their pesticide exposure. Moreover, they utilize a great array of different nesting sites—from the canopies of tall trees, to sites in the ground and in tree hollows. Some species have adapted to human habitation, making their nests in buildings and other structures. The growth cycle of stingless bees from egg to adult takes more or less seven weeks, with a larval period of two weeks, varying according to species (Nogueira-Neto 1997). Nonetheless, the food placed in any brood cell for a meliponine is stored previously and “matured” within the storage containers of pollen and honey. It is not the same food taken



Figure 3.2a

NEST ENTRANCE TO A HOLLOW TREE HOLDING A COLONY OF *MELIPONA BEECHEII*, IN MEXICO



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Figure 3.2b

HONEY STORAGE POT AND WORKER STINGLESS BEES IN THE NEST



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Figure 3.2c

HONEY REMOVAL USING A SUCTION DEVICE; HIVE OF *MELIPONA SUBNITIDA* IN BRAZIL



© D. W. Roubik

directly from flowers, which comprises the food of all other bees except honey bees and bumble bees (see Chapter 4), which also store honey and pollen.

The process of collecting food for provisioning brood cells is a route for female bee exposure to pesticides. During flights to collect pollen, nectar or oils used as food, or when foraging for plant or other material such as mud or resin to build nests, female bees can be exposed to toxic substances present in the environment. In addition, human-made toxins brought to the nest can contaminate and compromise larval development. Larvae that receive greater amounts of food, in species such as bumble bees (*Bombus*), and that subsequently become queens are likely to consume higher doses of pesticides. However, they may not be fed more toxin per unit of body weight.

Linkages between water collection and pesticide contamination are another area that needs further investigation. According to Ferry and Corbet (1996) water collection by bees may be for the benefit of the individual or the colony. Bees are less subject to desiccation than most terrestrial insects, due to their nectar diet. Bumble bee foragers rarely collect water for nest cooling, which is often done by honey bees. The possible contamination from such water collection needs to be investigated.

Table 3.2

PUBLISHED DATA ABOUT BROOD CARE AMONG BRAZILIAN WILD BEES

FAMILY	SPECIES	TIME SPENT IN CELL PROVISIONING	REFERENCE
APIDAE	<i>Xylocopa suspecta</i>	From 1 to 3 days	Camillo <i>et al.</i> , 1986
	<i>Eulaema nigrata</i>	From 6 to 9 hours for 11 days	Santos and Garófalo, 1994
	<i>Euglossa fimbriata</i>	From 2 to 5 days	Augusto and Garófalo, 2009
	<i>Euglossa (Euglossa) townsendi</i>	From 1 to 6 days	Augusto and Garófalo, 2004
	<i>Centris tarsata</i>	4 to 5 trips to collect pollen ($12,6 \pm 6,0$ min each) and 3-4 to collect oil ($7,4 \pm 2,1$ min each) to provisioning 1 cell	Aguiar and Garófalo, 2004
	<i>Centris analis</i>	4 to 11 trips for collect pollen ($14,3 \pm 11,5$ min each) and 2 to 8 to collect nectar ($8,8 \pm 10, 7$ min each) to provisioning 1 cell (time to collection is highly variable)	Vieira de Jesus and Garófalo, 2000
	<i>Centris trigonoides</i>	5 to 8 trips to collect pollen and nectar ($26,7 \pm 10,6$ min each) and 4 to 6 trips to collect oil ($16,8 \pm 8,5$ min each)	Aguiar <i>et al.</i> , 2006
	<i>Augochloropsis iris</i>	In the solitary phase, female spent 2 days to provisioning 1 cell	Coelho, 2002
	<i>Tetrapedia curvitaris</i>	8 to 17 trips to collect pollen ($37,5 \pm 16,4$ min each), 2 to 6 trips to collect oily substance ($24,0 \pm 15,4$ min each)	Camillo, 2005
	<i>Tetrapedia rugulosa</i>	13 to 19 trips to collect pollen ($28,1 \pm 18,5$ min each)	
	<i>Tetrapedia garofaloi</i>	5 to 9 trips to collect pollen ($37,6 \pm 11,2$ min each)	

WILD BEE NATURAL HISTORY IN RELATION TO NESTING RESOURCES

The nests of bees are the places where their young are reared and provided with food; nest cells serve to protect the immature stages of bees and their food resources. Nests can exist individually or as clusters of nests that are close together (Michener 2007).

Bees may construct their nest in burrows in the soil, in wood or pith, or in pre-existing cavities (inside tree hollows, abandoned nests of ants or termites, beetle or moth burrows in wood, or in human-made structures). Many materials are carried to the nests to construct those nests, like pieces of leaves, chewed leaf pulp, plant hairs, resin, pebbles, sand and mud. In some cases, certain plant materials may be cemented together with the use of saliva. Among the stingless bees, brood cells are built of wax mixed with resin (cerumen). The multiple layers of cerumen around the brood are called involucre.

While collecting materials to construct their nest, bees may be exposed to a wide array of agrochemicals. Many kinds of the collected material (mud, water, resin) may be contaminated with chemicals. Table 3.3 presents a summary of the nesting behavior of some Brazilian wild bees from the existing literature. The data presented illustrate construction localities, the material used and the Brazilian states where those species occur.



Table 3.3

PUBLISHED DATA ABOUT PLACES, CONSTRUCTION MATERIALS AND GEOGRAPHIC DISTRIBUTION OF BRAZILIAN WILD BEES

	PLACE	CONSTRUCTION MATERIAL	LOCATION (STATE)	REFERENCE
APIDAE				
<i>Bombus transversalis</i>	Soil nest (shallow depression on the forest floor)	Cut leaves	Acre, Amapá, Amazonas, Mato Grosso, Pará, Rondônia	Moure <i>et al.</i> , 2008; Taylor and Cameron, 2003
<i>Centris aenea</i>	Soil nest (clay or hard soil)	Oily substance and soil particules	Bahia, Ceará, Goiás, Maranhão, Mato Grosso, Minas Gerais, Pará, Rio Grande do Norte, São Paulo	Moure <i>et al.</i> , 2008; Aguiar and Gaglianone, 2003
<i>Centris dichrotricha</i>	Pre-existing cavities (wood)	Sand with oil or resin	Amazonas, Rondônia	Moure <i>et al.</i> , 2008; Morato <i>et al.</i> , 1999
<i>Centris tarsata</i>	Pre-existing cavities (wood; black cardboard)	Sand with oil or resin or sand with wax	Maranhão, Mato Grosso, Pará, Bahia	Moure <i>et al.</i> , 2008; Mendes and Rêgo, 2007; Aguiar and Garófalo, 2004; Silva <i>et al.</i> , 2001
<i>Centris trigonoides</i>	Pre-existing cavities (wood)	Sand with oil or resin	Bahia, Goiás, Pará, São Paulo	Moure <i>et al.</i> , 2008; Aguiar <i>et al.</i> , 2006
<i>Centris vittata</i>	Pre-existing cavities (wood)	Sand with oil or resin	Amazonas, Minas Gerais, Pará, São Paulo	Moure <i>et al.</i> , 2008; Pereira <i>et al.</i> , 1999
<i>Centris analis</i>	Pre-existing cavities (wood; black cardboard)	Pieces of wood; plant material and an oily substance	Amazonas, Ceará, Goiás, Mato Grosso, Paraná, Pará, São Paulo	Moure <i>et al.</i> , 2008; Morato <i>et al.</i> , 1999; Vieira de Jesus and Garófalo, 2000
<i>Centris bicornurta</i>	Pre-existing cavities (wood)	Pieces of wood	Amazonas, Pará, Piauí	Moure <i>et al.</i> , 2008; Morato <i>et al.</i> , 1999
<i>Centris terminate</i>	Pre-existing cavities (wood)	Sand with oil or resin; pieces of wood	Amazonas, Bahia, Pará	Moure <i>et al.</i> , 2008; Drummont <i>et al.</i> , 2008; Morato <i>et al.</i> , 1999
<i>Eufriesea smaragdina</i>	Pre-existing cavities (wood)	Pieces of wood and resin	Espírito Santo, Minas Gerais, Paraná, Santa Catarina, São Paulo	Kamke <i>et al.</i> , 2008; Moure <i>et al.</i> , 2008
<i>Euglossa townsendi</i>	Pre-existing cavities (wood)	Resin	Amazonas, Bahia, Espírito Santo, Minas Gerais, Pará, São Paulo	Moure <i>et al.</i> , 2008; Augusto and Garófalo, 2004
<i>Euglossa annectans</i>	Pre-existing cavities (wood)	Resin	Espírito Santo, Minas Gerais, Paraná, Rio de Janeiro, Santa Catarina, São Paulo	Garófalo <i>et al.</i> , 2008; Moure <i>et al.</i> , 2008
<i>Eulaema nigrata</i>	Pre-existing cavities (ant nest)	Mud, excrement and resin	Acre, Amapá, Amazonas, Bahia, Ceará, Distrito Federal, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Paraíba, Pará, Pernambuco, Piauí, Rio Grande do Norte, Rio de Janeiro, Rondônia, Roraima, Santa Catarina, São Paulo, Tocantins	Moure <i>et al.</i> , 2008; Santos and Garófalo, 1994
<i>Melipona quadrifaciata</i>	Pre-existing cavities (trees)	Cerumen (mixture of wax with resin) and mud	Mato Grosso do Sul, Minas Gerais, Paraná, Rio Grande do Sul, Rio de Janeiro, Santa Catarina, São Paulo	Moure <i>et al.</i> , 2008; Nogueira-Netto, 1997
<i>Melipona scutellaris</i>	Pre-existing cavities (trees)	Cerumen (mixture of wax with resin) and mud	Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Rio Grande do Norte, Sergipe	Moure <i>et al.</i> , 2008; Nogueira-Netto, 1997

follows on the next page →

	PLACE	CONSTRUCTION MATERIAL	LOCATION (STATE)	REFERENCE
APIDAE				
<i>Monoeca xanthopyga</i>	Soil nest (clay soil)	Oily substance	Paraná, Rio Grande do Sul, Santa Catarina	Moure <i>et al.</i> , 2008; Cunha and Blochtein, 2003
<i>Plebeia poecilochroa</i>	Pre-existing cavities; earth banks; human-made walls	Cerumen, wax, resin	Bahia, Espírito Santo, Minas Gerais, Pernambuco	Moure <i>et al.</i> , 2008; Drummond <i>et al.</i> , 1995
<i>Ptilothrix plumata</i>	Soil nest (clay soil)	Water and mud	Ceará, Maranhão, Mato Grosso, Pará, Pernambuco, Santa Catarina, São Paulo	Schilindwein <i>et al.</i> , 2009; Moure <i>et al.</i> , 2008;
<i>Xylocopa cearensis</i>	Branches of wood	No information	Bahia, Ceará, Goiás, Mato Grosso, Paraíba, Pará, Rio Grande do Norte	Moure <i>et al.</i> , 2008; Viana <i>et al.</i> , 2002
<i>Xylocopa frontalis</i>	Branches and stems	Sawdust	Acre, Alagoas, Amapá, Amazonas, Bahia, Ceará, Espírito Santo, Goiás, Maranhão, Mato Grosso, Minas Gerais, Paraná, Paraíba, Pará, Rio Grande do Sul, Rio de Janeiro, São Paulo	Pereira and Garófalo, 2010; Moure <i>et al.</i> , 2008
<i>Xylocopa grisescens</i>	Branches and stems	Sawdust	Alagoas, Amapá, Bahia, Ceará, Goiás, Maranhão, Mato Grosso, Minas Gerais, Paraíba, Pará, Pernambuco, Piauí, Rio Grande do Norte, Sergipe, São Paulo	Pereira and Garófalo, 2010; Moure <i>et al.</i> , 2008
<i>Xylocopa ordinaria</i>	Branches and stems	Sawdust	Espírito Santo, Mato Grosso do Sul, Rio Grande do Sul, Rio de Janeiro	Bernadino and Gaglianone, 2008; Moure <i>et al.</i> , 2008
<i>Xylocopa subcyanea</i>	Branches and stems	No information	Alagoas, Bahia, Espírito Santo, Goiás, Mato Grosso, Minas Gerais, Paraná, Pará, São Paulo	Moure <i>et al.</i> , 2008; Silva and Viana, 2002
<i>Xylocopa suspecta</i>	Branches and stems (dead and dry)	Sawdust	Bahia, Espírito Santo, Mato Grosso, Minas Gerais, Paraíba, Pará, Pernambuco, Rio Grande do Sul, Rio de Janeiro, São Paulo	Moure <i>et al.</i> , 2008; Camillo <i>et al.</i> , 1986
ANDRENIDAE				
<i>Cephalurgus anomalus</i>	Soil nest (unshedded horizontal ground)	Fine and homogeneous sediment and pebbles	Minas Gerais, São Paulo	Moure <i>et al.</i> , 2008; Gaglianone, 2000
MEGACHILIDAE				
<i>Anthodiocetes lunatus</i>	Pre-existing cavities (wood)	Plant resin with pieces of wood	Acre, Amazonas, Pará, Paraíba	Moure <i>et al.</i> , 2008; Camarotti-de-Lima and Martins, 2005
<i>Anthodiocetes megachiloides</i>	Pre-existing cavities (wood or mud)	Plant resin	Bahia, Ceará, Mato Grosso, Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina, São Paulo	Alves-dos-Santos, 2004; Moure <i>et al.</i> , 2008
<i>Anthodiocetes moratoi</i>	Pre-existing cavities (wood)	Plant resin with pieces of wood	Amazonas	Moure <i>et al.</i> , 2008; Morato, 2001
<i>Megachile habilis</i>	Pre-existing cavities (wood)	Pieces of leaves	Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná	Moure <i>et al.</i> , 2008; Laroca <i>et al.</i> , 1987
<i>Megachile pseudanthidioides</i>	Pre-existing cavities (wood)	Pieces of leaves, flower petals and mud	Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina, São Paulo	Moure <i>et al.</i> , 2008; Zillikens and Steiner, 2004
<i>Megachile orbiculata</i>	Pre-existing cavities (wood)	Pieces of leaves	Acre, Amozonas, Bahia, Mato Grosso, Pará	Moure <i>et al.</i> , 2008; Morato, 2003
<i>Megachile anthidioides</i>	Pre-existing cavities (cardboard)	Pieces of leaves	Minas Gerais	Sabino and Antonini, 2011

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	PLACE	CONSTRUCTION MATERIAL	LOCATION (STATE)	REFERENCE
COLLETIDAE				
<i>Peditomorpha brunerii</i>	Soil nest (unshaded horizontal ground)	Fine and homogeneous sediment and pebbles	São Paulo	Gaglianone, 2000
HALICTIDAE				
<i>Augochloropsis iris</i>	Soil nest	No information	Minas Gerais, Paraná, São Paulo	Moure <i>et al.</i> , 2008; Coelho, 2002
<i>Caenohalictus curtipectus</i>	Soil nest (well-shaded vertical road-side bank of rather moist)	Consistent soil; glandular secretion	Paraná, Rio de Janeiro, São Paulo	Moure <i>et al.</i> , 2008; Sakagami and Moure, 1967
<i>Lasioglossum seabrai</i>	Soil nest (well-shaded vertical road-side bank of rather moist)	Consistent soil; glandular secretion	Paraná	Sakagami and Moure, 1967
<i>Megalopta aegis</i>	Dead wood (stems, lianas, and branches)	Pith and oil	Goiás, São Paulo	Santos <i>et al.</i> , 2010; Moure <i>et al.</i> , 2008
<i>Megalopta guimaraesi</i>	Dead wood (stems, lianas, and branches)	Pith and oil	São Paulo	Santos <i>et al.</i> , 2010
<i>Neocorynura polybioid</i>	Soil nest (well-shaded vertical road-side bank of rather moist)	Consistent soil; glandular secretion	Minas Gerais, Paraná, Rio de Janeiro, São Paulo	Moure <i>et al.</i> , 2008; Sakagami and Moure, 1967
<i>Pseudagapostemon divaricatus</i>	Soil nest (well-shaded vertical road-side bank of rather moist)	Consistent soil; glandular secretion	Paraná	Sakagami and Moure, 1967
<i>Rhinocorynura inflaticeps</i>	Soil nest (well-shaded vertical road-side bank of rather moist)	Consistent soil; glandular secretion	Minas Gerais, Paraná, Santa Catarina, São Paulo	Moure <i>et al.</i> , 2008; Eickwort and Sakagami, 1979

APPLICATION AND TOXICOLOGY

Here, it is suggested that empirical studies in Brazilian wild bee natural history could help predict pesticide exposure (Figure 3.3). Several methods have been designed to evaluate the toxic effects of pesticides. In general, toxicological analysis are based on three main steps: (a) laboratory tests determining acute toxicity (topical and by ingestion), survival (time elapsed until death) and behavioral alteration; (b) semi-field tests; and (c) field tests, evaluating the mortality of bees/larvae/pupae, foraging activity, colony development and general behavior (OEPP/EPP0 2001) (Figure 3.4).

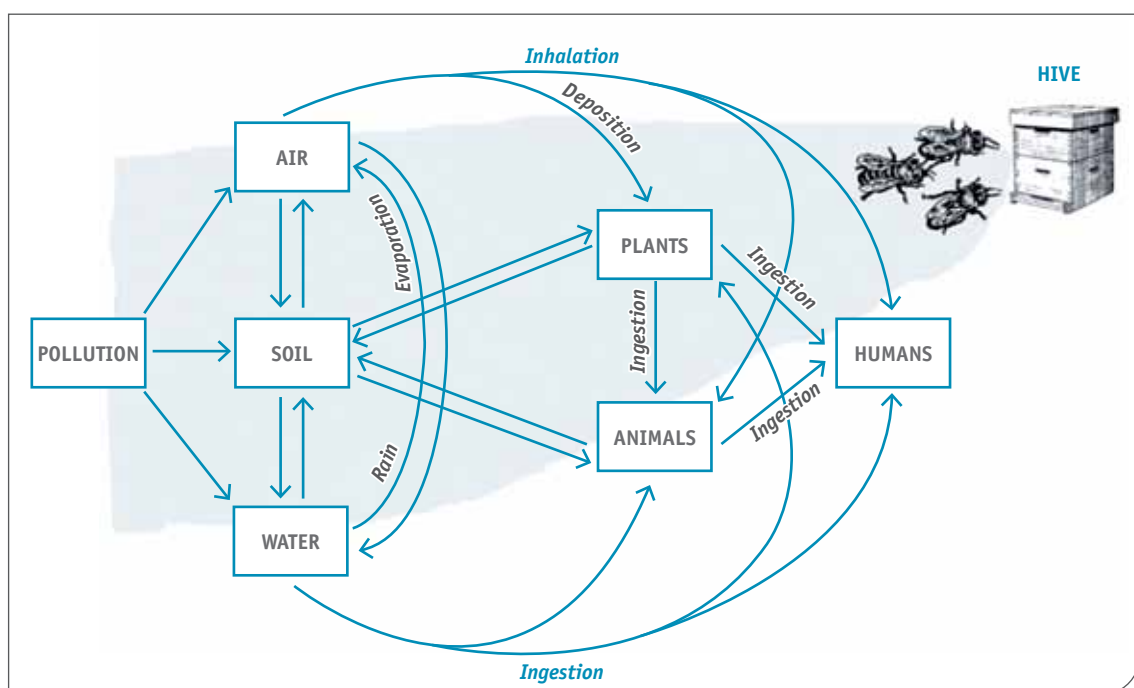
In 2007, Aupinel demonstrated the applicability of acute toxicity studies using an *in vitro* method for rearing larvae of *Apis*. Brodschneider *et al.* (2009) used a similar technique to evaluate toxicity impact on the flight capacity of adult *Apis* reared in the laboratory, and further work has been performed using more sensitive methods (Dai *et al.* 2012). These impressive advances in toxicology assay for *Apis* draw attention to the large technical and scientific deficiencies in the development of similar techniques for wild bee species.

Generally, the smaller body size of meliponine bees (for example 7-10 mg for many *Scaptotrigona* and 40-80 mg for many *Melipona*) as compared to 70-80 mg for Africanized honey bees means that Meliponini have a larger body surface area, making them more susceptible to contact poisoning from insecticides. For example, a lethal dose (LD_{50}) of fipronil to *A. mellifera* is, on average, 5.8 nanograms per bee (Carvalho *et al.* 2013), and for *Scaptotrigona postica* it is only 0.54 nanograms (Jacob *et al.* 2013). Similarly, *Bombus terrestris*, considerably more massive than *A. mellifera*, is 60 to 90 times more tolerant to deltamethrin than *A. mellifera* (Tasei 2002). It is worth noting, however, that body size is not the only factor responsible for susceptibility to toxins, but also health, nutritional condition and enzymatic systems, among other variables (Stenersen 2004).

The challenge of designing toxicity tests in the laboratory is to identify the best manner to extrapolate the data obtained to field conditions (Stark *et al.* 1995). Several approaches have been designed for this purpose, including the “hazard ratio” proposed by Felton *et al.* (1986), “selective ratio” (Croft 1990) and the “sequential testing scheme” of Johansen and Mayer (1990). While a considerable portion of investigation of pesticide impact on bees focuses

Figure 3.3

DIFFERENT PATHWAYS BY WHICH BEES COULD BE POISONED BY PESTICIDE RESIDUES IN THE ENVIRONMENT (FROM PORRINI *ET AL.* 2003)



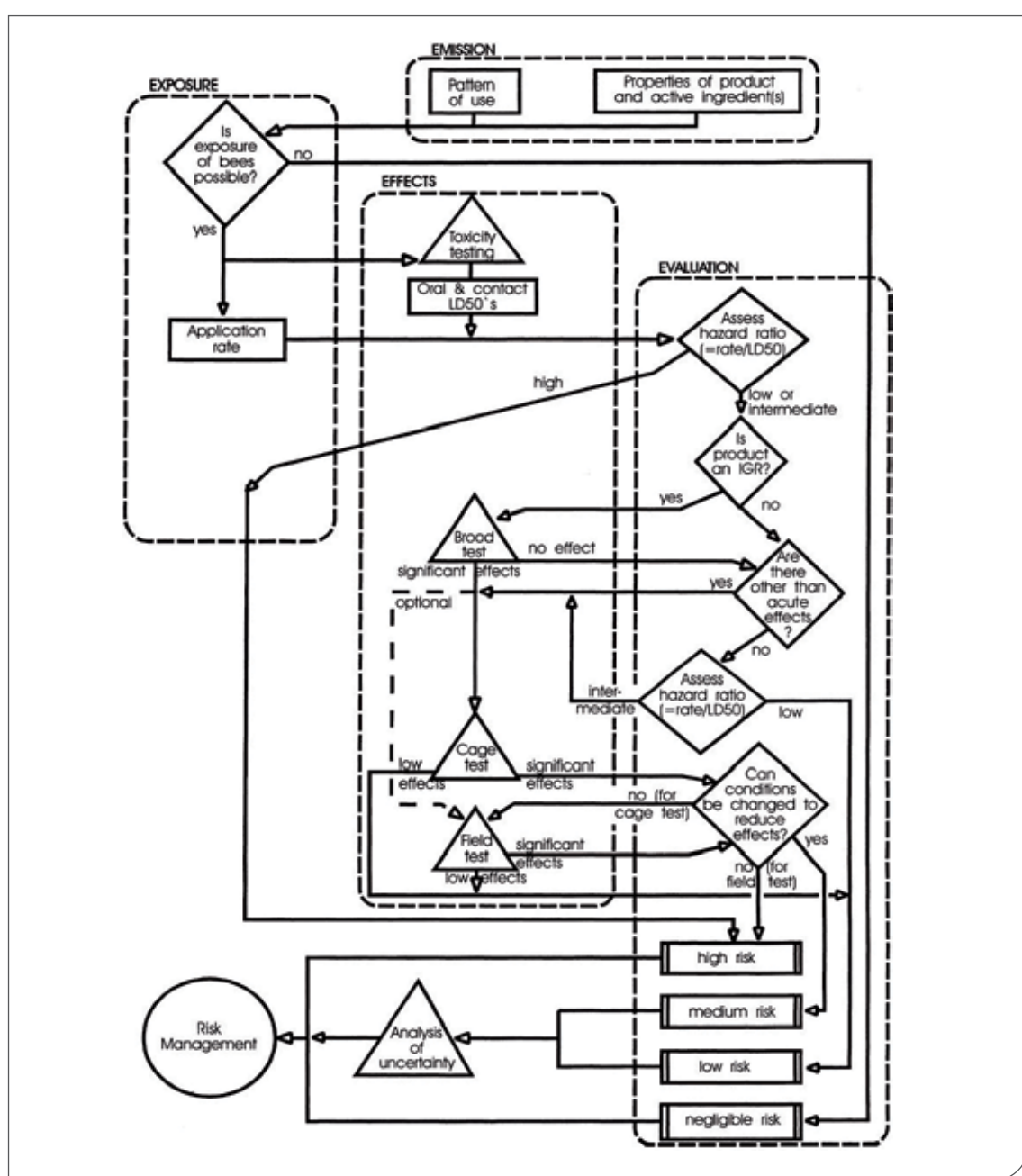
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on mortality, the sub-lethal effects of pesticides should also be taken into account. Important aspects of bee biology and behavior that may be included are the "division of labor" in colonies, foraging, colony development, nestmate recognition, larval/adult behavior, and flight capacity (Vandame *et al.* 1995; Thompson 2003).

Figure 3.4

SCHEME FOR EVALUATION OF PLANT PROTECTION CHEMICALS ON HONEY BEES



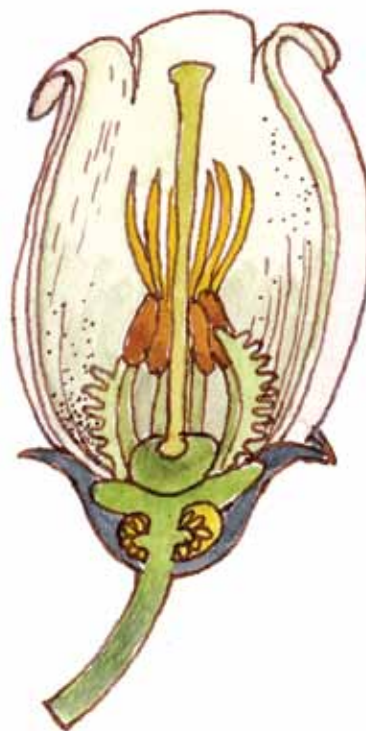
There is currently great public concern about the undesirable effects of plant protection products on beneficial organisms and human health. Yet it remains difficult to obtain conclusive and widely consistent evidence about pesticide effect on pollinator communities, as such effects may be very context-specific. Recently, Brittain *et al.* (2010) concluded that there are no proven differences in the availability of floral resources, abundance of bee species and pollination between areas cultivated in a conventional manner, including pesticide application, and those utilizing organic systems. In this context, the surrounding landscape may nonetheless play a major role, with benefits from the creation and maintenance of habitat corridors that allow transit between natural environments relatively free of contamination.

An ecologically appropriate strategy in pest control is integrated management (e.g. Integrated Pest Management, or IPM), primarily making use of natural or biological controls and using selective pesticides only when necessary (Kogan 1998). The use of pesticides that negatively affect beneficial organisms ultimately works against producing healthier food and protecting the environment (Croft 1990). In cases where the pesticide use is necessary, the chemical pesticide properties and application practices must be judged, to minimize toxic exposure (see Annex 1 and 2). For example, the simultaneous use of insecticide (pyrethroid) and fungicide (triazole), which act as synergists, can induce death of up to 67.5 percent more honey bee individuals, in comparison with the agrochemicals applied singly (Colin and Belzunces 1992).

An effective mitigation measure to reduce the risk of pesticide exposure is to plan the timing of application for periods of the day (or night) when bees are not foraging. Byrne and Waller (1990) conclude that night time applications of dimethoate on citrus reduce bee mortality by half, compared to daytime applications. However, night time applications still result in mortality levels 3.76 times higher than that in a control treatment. Planning an optimal time of application is not sufficient to protect bees. Chemicals with low toxicity and residue, and those with selective capacity against target pests, are useful but usually more costly.

The remarkable bee diversity in the Neotropics is an important part of ecosystem and human health. Protection of those bees from incorrect practices and pesticide use is therefore a priority issue. Mitigation measures are needed, including:

- (1) increasing investment in studies on the biology, physiology, behavior and management of bees, to provide knowledge that can be used to enhance pollination services.
- (2) developing new evaluation techniques specific for wild bees that assess the lethal and sub-lethal effects of plant protection products.
- (3) increasing awareness in all the sectors of production (farmers, beekeepers and industrialists) for developing agricultural management strategies that are less destructive to bees.



Pollinator-dependent crops: blueberry above, mango on left lower, blueberry, again, on lower right.

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APPENDIX

GENERAL NATURAL HISTORY OF BRAZILIAN WILD BEES AND THEIR IMPORTANCE IN POLLINATION

SHORT-TONGUED BEES

The families Andrenidae, Colletidae and Halictidae are short-tongued bees; they have relatively short but often wide tongues used to imbibe nectar and other liquids.

Andrenidae

During the rainy season in northeastern Brazil, bees of the subfamily Panurginae are found in areas covered by Caatinga vegetation. Some species have also been recorded on the coast. Panurgine bees seem to forage exclusively on small shrubs and herbs, not occurring in tropical rain forest habitats (Schlindwein 2003). Ruz and Rozen (1993, 1995) studied the behavior of these bees and described the small bee species found in the Catamarca province of Chile and Argentina.

Colletidae

Several species of Colletidae are considered to be oligoleges, or flower visitors that specialize on the pollen of a few related plant species (Kearns and Inouye 1993). Oligolectic bees frequently have behavior and other adaptations to transport particular kinds of pollen grains. The long branched hairs observed in most Colletinae is a morphological adaptation shared with many other bees. Another is seen in the short scopal structures of *Perditomorpha brunerii* that allow large pollen grains to be transported (Gaglianone 2000). Colletid bees are solitary and the most common genera are *Colletes*, *Hylaeus*, *Ptiloglossa*, *Tetraglossula* and *Perditomorpha* (Imperatriz-Fonseca and Santos 2011).



Halictidae

Halictidae is one of the most diverse bee families in Brazil. Bees of this family often have bright metallic colour that can be green, blue, red or black (Imperatriz-Fonseca and Alves dos Santos 2011). There are different levels of sociality in this family, and also solitary bees. An important group is the augochlorine bees, which visit a wide variety of flowers in forested and open habitats over a wide area—from coastal lowlands to the high Andes (Eickwort 1969).

LONG-TONGUED BEES

The families Megachilidae and Apidae constitute the long-tongued bees, and are often the most abundant bees in nature.

Megachilidae

Megachilidae is well represented in Brazil primarily by bees of the genus *Megachile*, which has 32 subgenera (Moure *et al.* 2008). Some species construct their nests with cut pieces of leaves and other plant material (Michener 2000, 2007). For example, Zillikens and Steiner (2004) provided the first description of the nests and lifecycle for the subgenus *Chrysosarus*. They set up trap nests (drilled tunnels in wood) in a survey in the state of Santa Catarina, Brazil and these bees used leaves and petals to construct their nests during two annual generations.

Apidae

An interesting example of this group is the genus *Xylocopa* (carpenter bees). *Xylocopa* are found in several states in Brazil across different latitudes, from Rio Grande do Sul to Acre and Amapá. These bees are sometimes solitary but mainly social, with multiple females, and sometimes males, in a nest or group of nests in a tree trunk or branch (see Chapter 7). In Brazil they are known as ‘mamangavas’ or ‘mamangabas’. Further general discussion concerning these large bees is given by Gikungu in the final chapter of this book—the genus occurs worldwide and has over 300 species. Another important genus is *Centris*, of which Silveira and Campos (1995) identified 36 species in the state of Minas Gerais alone.

Apinae is the largest subfamily encountered in Brazil and includes the Africanized honey bee (Apini), the bumble bees (Bombini), the stingless bees (Meliponini), and the orchid bees (Euglossini). They have generalist flower visitation habits and year-round activity, with the first three groups exhibiting advanced social behavior. Common bumble bee species in Brazil include *Bombus morio*, *B. atratus* and *B. brasiliensis* (Imperatriz-Fonseca and Alves dos Santos 2011). Augusto and Garofalo (2004) described nest behavior of *Euglossa townsendi*, a species in which

two or more females of different or the same generation share a nest. Euglossine males visit orchids and other natural resources to collect fragrances (Ramirez *et al.* 2011; Imperatriz-Fonseca and Alves dos Santos 2011).

The Meliponini are eusocial bees known as stingless bees because no female or worker bee has a functional sting. There is a remarkable richness of these bee species in the Neotropics (Freitas *et al.* 2009). According to Kerr *et al.* (1996) stingless bees are responsible for 40 to 90 percent of the pollination of wild plant species in different tropical ecosystems. The stingless bees often visit flowers in the upper canopy and in their absence the communities of tropical rain forest trees would be extensively modified (Wille 1983). The meliponine bees are considered floral generalists, collecting pollen and nectar from a wide variety of plant species. However, studies performed with *M. scutellaris* (Ramalho *et al.* 2007) show that according to need, the foragers may exhibit a temporary floral fidelity (Ramalho *et al.* 1994, 1998). This behavior is the result of communication skills among individuals demonstrated by species of stingless bees. Several species of Meliponini of the Caatinga are endemics, which are endangered by human activity. Honey and pollen stores of the colonies of many *Melipona* are harvested by honey-hunters and local beekeepers. Deforestation and unsuitable management of natural and agroecosystem resources are also threatening some of these species, which are already rare (Martins 2002).



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Stingless bees at the entrance to their nest in Laikipia, Kenya. Many species of stingless bees construct tubes from resin like that shown here.



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Top: Bombus morrisoni; middle: Bombus impatiens; bottom: Bombus pennsylvanicus.

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CHAPTER 4

BUMBLE BEES: NATURAL HISTORY AND PESTICIDE EXPOSURE ROUTES

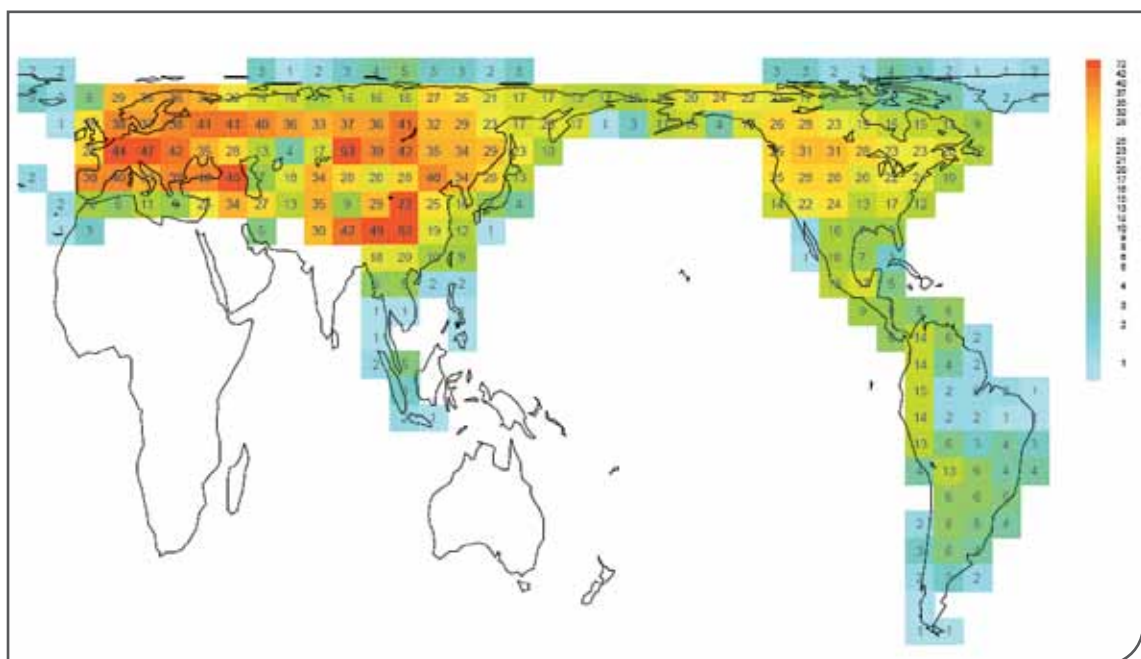
Sheila Colla

AN INTRODUCTION TO BUMBLE BEES

Bumble bees are large and brightly coloured bees. Because they are found in relatively high abundance in very populated regions of the world, they are fairly well known. They belong to a single genus, *Bombus*, in the family Apidae, although some are parasites, in the subgenus *Psithyrus*. Globally there are approximately 250 species and the highest diversity is in northern temperate regions (Figure 4.1).

Figure 4.1

GLOBAL BUMBLE BEE SPECIES DIVERSITY (WILLIAMS 1998)



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Bumble bees are eusocial, with a reproductive caste or queen, and a sterile caste of workers. They exhibit cooperative brood care, and they feed primarily on pollen and nectar. In the temperate zone, mated queens that have hibernated in a sheltered niche resume activity in the spring and gather resources to initiate a nest. When the first brood of workers hatch they take over nest care, tending larvae and foraging while the queen continues to lay eggs. The colony continues to grow as foragers bring back pollen and nectar and more workers hatch. Near the end of the colony cycle, the queen lays eggs that become males and new queens. These reproductive individuals then leave the nest; they mate with conspecifics and the mated queens then hibernate, emerging as active females in the following spring. In the tropics, on the other hand, certain tropical *Bombus* may continue to live as a colony for several years.

With their generally long foraging season, bumble bees are generalists – foraging on many different plant species. However, bumble bees will select nectar flowers based upon their tongue length, the corresponding length of the floral corolla—the tube through which they reach the nectar—and food reward value. Individual bee handling time and cognitive abilities have also been found to affect foraging success.

Bumble bees weigh between 0.04 g and 0.85 g. Queens are normally the largest, but this varies by species. Males weigh approximately the same as a large worker. Additionally, in some species, workers produced early in the season tend to be smaller than their sisters produced later on, when food resources are more abundant.

Bumble bee declines for certain species have been noted in parts of Europe, Asia and North America (Williams and Osbourne 2009). The causes of these declines are still being studied, but hypotheses include habitat loss (loss of suitable forage plant species and nesting sites), disease, pesticide use and climate change. Some declining species are those thought to specialize on long, tubular flowers for nectar, such as those in Papilionoideae of the Fabaceae. Bumble bees are important pollinators of many native temperate flowering plants and certain crops. They are particularly effective at pollinating crops in greenhouses. Managed bumble bees are increasingly being used to support agricultural and horticultural production. Indeed, over one million bumble bee colonies of different species were sold worldwide in 2006, primarily for greenhouse fruit and vegetable production. Although sales have been largely to pollinate tomatoes, there are more and more for commercial orchards and seed production (Velthuis and Doorn 2006).

WILD BEE NATURAL HISTORY IN RELATION TO FORAGING

Assessment of pesticide exposure risks to bumble bees requires quantifying the duration and rate of possible exposure. Crops where pesticide sprays may be applied and from which bumble bees

are known to forage include raspberry, strawberry, blueberry, cranberry, stone fruits, sunflower, tomato, cucumber, sweet peppers, string beans, soybeans, peas, watermelon, rose-hips and cotton. Some relevant average statistics, extracted from the literature, are presented below.

One bumble bee on a favorable ten-hour day could visit 6,000 flowers and on an average four-to five-hour day visit 2 500 to 3 000 flowers (Macfarlane 1995). The average foraging bout for a worker bee is four to seven minutes. Per foraging bout, worker bees collect 1.44 to 27.33 mg of pollen (Plowright *et al.* 1993) and approximately 70 μ L (one milliliter (ml) is equal to 1000 μ L) of nectar. Loads of pollen and nectar can reach up to 20 percent and 90 percent, respectively, of the bee's body weight (Benton 2006), thus larger bees tend to bring back larger loads.

A study by Müller and Schmid-Hempel (1992) found that individual bees spend 14 to 30 days foraging, and colonies have foragers for 50 to 150 days. The season of activity varies with latitude, elevation and species. Toward the poles, the summer daylight hours are extended, thus daily foraging periods are longer. As already mentioned, temperate latitudes have bumble bees that begin the summer colony cycle when mated queens emerge from hibernation in the spring. On days with low winds and little or no precipitation, workers generally forage at 5 to 30°C, dawn to dusk, but this also varies with weather, latitude and species. According to Macfarlane (1995), bumble bee foraging activity increases nine-fold from the onset of foraging at about 13°C, to 22°C later in the day. At about 27°C bumble bee foragers in the field will stabilize and then decline in the middle of the day as temperatures surpass 30°C. In a Wisconsin, USA, study, 85 percent of bumble bee foraging on cranberry flowers occurred between 10 a.m. and 7 p.m. (Macfarlane 1995). A recent study performed in Central Europe (Hagen *et al.* 2011) obtained maximum flight distances from the nest of bumble bees. These reach 2.5 km, 1.9 km and 1.3 km for *Bombus terrestris* (workers), *Bombus ruderatus* (workers), and *Bombus hortorum* (young queens), respectively. Additionally, estimated home range sizes are 0.25–43.53 ha (Hagen *et al.* 2011).

WILD BEE NATURAL HISTORY IN RELATION TO BROOD CARE

Bees, unlike most other insects which merely lay eggs on hosts or food plants, have an added complication —and possible pesticide exposure route: they provide food for their offspring. In fact, numerous studies have suggested social bees, with continued brood care, are quite likely to be more susceptible to pesticide exposure than solitary insects (Brittain and Potts 2011). Thus, an assessment of pesticide exposure needs to consider the quantities of pesticides that may contaminate food resources that adult female bees use to feed the brood, versus what is self-consumed. It is often easier to sample and then study the food resources in bee nests than those consumed by the foraging adult.



The feeding of larvae differs between species in one of two ways. “Pocket-makers” place pollen in a pocket within the brood area and the larvae feed from a common pollen deposit as they develop. “Pollen-storers”, on the other hand, make separate pollen storage containers or “pots”, from which pollen is then doled out to the larva individually, as needed, through a hole in the brood cell (Benton 2006).

The average weight of pollen brought back to the nest per bee-day is, according to one study, 15.25 to 30.86 mg (Plowright *et al.* 1993). The average number of pollen grains consumed by a worker larva has been measured at 8.5 million, and by a queen larva at 22 million (Ribeiro 1994). The percentage of pollen mass to body mass for worker and male larvae is higher (medians: 25.58 and 25.12 percent, for workers and males, respectively) than for queen larvae (median: 10.12 percent) (Figure 4.2). This finding indicates that developing queens ingest more nectar (therefore sugar) and less pollen than the other bees. Their total body mass receives a large contribution from sugar turned into fat (important as a reserve for winter hibernation) from carbohydrates.

The ratio of males, workers and queens produced by the founding queen varies widely e.g. 4 to 218 workers, 1 to 74 males, 0 to 125 queens in a study of 36 *B. lucorum* colonies (Müller and Schmid-Hempel 1992).

WILD BEE NATURAL HISTORY IN RELATION TO NESTING RESOURCES

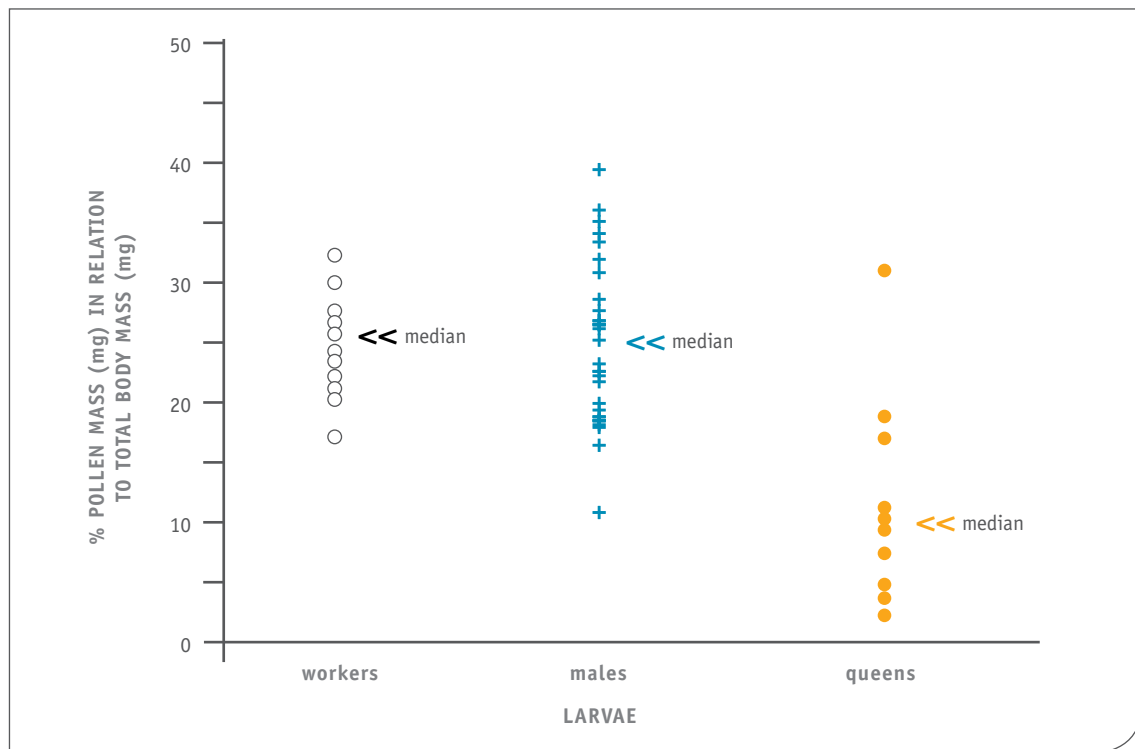
Because bees must have a nest in which brood are reared, they may also be exposed to pesticides from the nesting resources they use. Depending on the species, nests can be below ground (usually in empty rodent burrows), above ground (protected by tall grass, roots or vegetation) or in the leaves and stems of trees. Some species having above ground colonies require tall grasses for protection.

CONCLUSIONS

In reviewing the possible pesticide exposure routes for bumble bees, it is evident that there are critical gaps in knowledge. For example, the proportion of pollen and nectar self-consumed by the worker and the proportion brought back to the colony are unknown. Ecological differences between species may allow for differential impacts of pesticides, as in pollen-storing species vs. pocket-making species. Pocket-makers may have only a group of larvae affected by contaminated pollen, whereas pollen-storers feed all developing larvae of the colony from the same pollen mass. What is also needed is information on the mixing and maturation of pollen, in either pocket-makers or pollen-storers, before it is fed to larvae. Within the pollen stores of both

Figure 4.2

PERCENTAGE OF POLLEN MASS IN RELATION TO TOTAL MATURE LARVA BODY MASS FOR WORKER, MALE AND QUEEN LARVAE OF *BOMBUS TERRESTRIS*



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solitary and social bees, there are microbes that can digest and even detoxify pollen, but their nature and ecology are scarcely known.

The majority of studies perform experiments on actively managed bumble bee species (*B. terrestris* & *B. impatiens*), which may be more tolerant to stress than other species. However, Wu *et al.* (2010) show that pesticides do not affect all species equally. Thus, studies should be done on an array of species, not just the captive bred, commercialized bees, in order to better understand the effects of pesticides on wild bumble bees. The stage at which the colony is most vulnerable to pesticide exposure is likely to be when the spring queen is the sole forager. Bumble bee species that nest above ground may be more susceptible to direct pesticide exposure.

Field testing has shown that pesticide levels considered safe in laboratory toxicity tests can nonetheless be detrimental to foraging bumble bees (Mommaerts *et al.* 2010). Likewise, sub-lethal effects on cognitive abilities (which affect orientation and navigation in the field), flower handling time, colony and larval growth are likely to be unfavourable to overall reproductive success for all bumble bee species (e.g. Gradish *et al.* 2010; Tasei *et al.* 2008;



Thompson 2003). For example, Morandin and Winston (2003) found the time spent by *Bombus impatiens* to access artificial flowers increased after exposure to high levels (30 ppb) of imidacloprid in pollen.

When developing management practices to reduce risks, some specific recommendations are:

- Perform experiments to test effects of pesticides on bumble bees in natural settings, i.e. outside of a controlled cage or laboratory (Thompson 2003), which include environmental stressors, such as changes in food availability and weather conditions, and which require bees to forage normally.
- Consider both castes, because variation in growth requirements may lead to differential susceptibility between castes, as reviewed by Mommaerts and Smagghe (2011).
- Examine synergistic effects of pesticides with diseases, microbes, endosymbionts, and pollen or nectar surplus or shortage.

Figure 4.3

***BOMBUS IMPATIENS* FORAGING ON THISTLE**



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A male of the common eastern (N. American) bumblebee, Bombus impatiens.



Top: females of blue orchard bees; bottom: female leafcutter bee.

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CHAPTER 5

PESTICIDE EXPOSURE ROUTES FOR WILD BEES: THE LEAFCUTTER AND MASON BEE GROUP— MEGACHILIDAE

Barbara Gemmill-Herren
Erhard Strohm

(With contributions from Cory S. Sheffield and Sabine Radmacher)

NATURAL HISTORY OF THE MEGACHILIDAE AND THEIR IMPORTANCE IN POLLINATION

Megachilidae are the second largest bee family in terms of described species and are highly diverse with respect to life-history traits (Litman *et al.* 2011; Gonzalez *et al.* 2012). Those bees include excavators that dig nest burrows in the soil and/or decomposing wood; those that nest in pre-existing cavities in wood, plant stems, and even abandoned snail shells and almost any other natural or artificial cavity; masons that construct nests on exposed or concealed surfaces (e.g., rocks, twigs), using mud or resin; and cleptoparasites —bees that collect no larval food provisions from flowers, but instead lay eggs in the nests of their host(s).

Among the Megachilidae are the leafcutter bees and the mason or carder bees, which include some important managed crop pollinators. One example is the alfalfa leafcutter bee *Megachile rotundata*, originally from the Mediterranean region and the Middle East. After its accidental introduction into North America around 1940 (Krombein 1948) it was found to be of great importance in the pollination of alfalfa flowers to produce seed (Hobbs and Lilly 1954; Bohart 1962), for which it is now managed. More recently, it has been developed as a managed pollinator of lowbush blueberry in northeastern North America (Stubbs *et al.* 1996; Sheffield 2008). *Osmia rufa*, the red mason bee, is very abundant throughout Europe



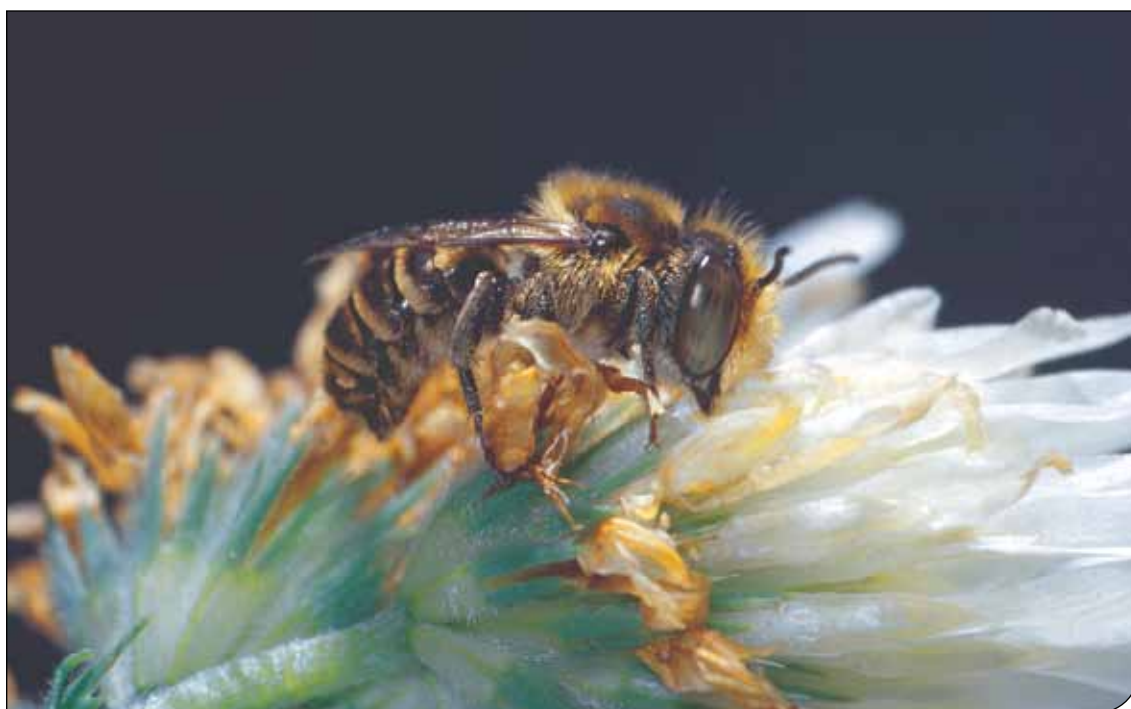
(Westrich 1989) and *Osmia lignaria*, the orchard mason bee, is a very promising North American solitary bee for pollinating tree-fruit crops (Bosch and Kemp 2001). Here, the primary focus is on just these three species because their role in crop pollination is well known and better studied than among other megachilids.

The subfamily Megachilinae (excluding the tribe Lithurgini) is also unique among bees in that, unlike bees that apply nest linings, most Megachilinae must collect all their nesting materials (Michener 2007; Litman *et al.* 2011).

Although the life history trait of collecting all necessary nesting materials has contributed to the diversity and large geographic distribution of the Megachilidae, it also provides additional exposure routes to agrochemicals. Megachilid bees harvest not just nectar and pollen but also materials to line their nests and construct cell partitions, which include leaves that are cut into small pieces and/or flower petals for construction of “thimble-shaped” brood cells. Other megachilids use mud, and therefore mix water with earth to make it, or use pebbles, resin, glandular secretions or mixtures of these, masticated leaves or “leaf pulp”, and plant hairs or “trichomes”. Thus, because such materials are taken from the habitats surrounding their nesting sites, bees may be exposed to pesticides that reach those bee resources.

Figure 5.1

MEGACHILE ROTUNDATA



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Figure 5.2a

LEAFCUTTER BEE CUTTING AWAY A PORTION OF CHILLI PEPPER (*CAPSICUM*) LEAF, KENYA



© D. Martins

Figure 5.2b

LEAFCUTTER BEE CARRYING A PORTION OF CHILLI PEPPER (*CAPSICUM*) LEAF BACK TO ITS NEST, KENYA



© D. Martins

Figure 5.2c

CHILLI PEPPER (*CAPSICUM*) LEAF WITH PIECES OF LEAF REMOVED BY A LEAFCUTTER BEE, KENYA



© D. Martins



Management of megachilid bees for pollination is different from management of other species (i.e. honey bees, bumble bees, stingless bees) in that once nests are established within or adjacent to cropping systems, megachilids should not be moved (Bosch and Kemp 2001; Sheffield *et al.* 2008). Relocation of established nesting sites greatly reduces the overall fecundity of nesting populations, a large concern if the bees are being bred or propagated. Nesting bees may remain active for much longer than a particular crop flowering period, which may cause reduced production if alternative food resources are not available (Sheffield *et al.* 2008). However, because chemical applications for pest control are usually performed before and after flowering, bee populations nesting near the crop are at risk of exposure for an extended period.

In this review of pesticide exposure and megachilid bees, it is important to begin with an assessment of the overall size of the bee, as this is thought to be critical to pesticide vulnerabilities (Tables 5.1 and 5.2). It is suggested, for example, that smaller bees will receive a relatively higher dose of pesticides on a contact exposure basis than would larger bees. They have a greater surface area to volume ratio. Since the foraging range of smaller bees is less than that of larger bees, a smaller bee occupying an area in or near pesticide-treated fields may have a larger portion of their foraging area contaminated with pesticides than would a larger bee (Fischer and Moriarty 2011).

Table 5.1

CHARACTERISTICS OF MALE AND FEMALE *MEGACHILE ROTUNDATA* (F.); *OSMIA LIGNARIA* AND *O. BICORNIS* (FORMERLY KNOWN AS *O. RUFA*)

LEAFCUTTER BEES, BODY LENGTH	FEMALE	MALE
<i>Megachile rotundata</i>	8–10 mm	7–9 mm
<i>Osmia lignaria</i>	10–11 mm	8–10 mm
<i>Osmia bicornis</i> (<i>rufa</i>)	10–13 mm	8–10 mm

Table 5.2

BODY MASS CALCULATIONS (*MEGACHILE ROTUNDATA*; *OSMIA LIGNARIA*; *OSMIA BICORNIS* (*RUFA*); AND *OSMIA BICORNIS* (*RUFA*) COCOONS)

LEAFCUTTER BEES, BODY MASS	FEMALE	MALE
<i>Megachile rotundata</i> (Germany: Klostermeyer 1972)	35 mg avg	(no information)
<i>Osmia lignaria</i> (Canada: Sheffield 2008b)	130 mg avg	75 mg avg
<i>Osmia bicornis</i> (<i>rufa</i>) (Germany: Strohm <i>et al.</i> 2002)	99.9 ± 0.05 mg avg ± SD, with a decrease about 3 weeks after the onset of nesting activity, to about 82% of the initial value	(no information)
<i>Osmia bicornis</i> (<i>rufa</i>) cocoon weight (Germany: Radmacher and Strohm 2010)	35–135 mg	25–85 mg

SEASONAL PATTERNS

Alfalfa leafcutter bee: *Megachile* fly in summer and spend the winter as mature larvae (Sheffield 2008). At the beginning of summer, males of *M. rotundata* emerge from the nests a few days before the females and remain nearby, feeding on floral nectar. Mating occurs when females emerge from their nesting places (Pinzauti 2000). Males can mate with more than one female and generally remain near nesting sites for about two weeks, spending the nights in protected sites (crevices, under lumps of earth or other sheltered places). In order to carry out their activities (feeding, flight, copulation, etc.) males forage nectar and pollinate flowers. Although many alfalfa leafcutter bees will nest gregariously in a common site, each female builds its own tunnel, and the male, as among bees in general, is not involved in nesting (Pinzauti 2000). Females provision their nests and lay an egg on each provision mass before sealing the cell.

The egg-laying activity of female *M. rotundata* lasts 30 to 40 days. The larvae feed continuously on the provision mass and go through different larval stages until October (in Europe), when they build a silky cocoon where they spend the cold winter as a “prepupa”, in diapause. The next spring, diapause is broken and the immature bees return to their normal metabolic activity, pupate and emerge at the beginning of summer (Pinzauti 2000).

Red mason bee: *Osmia* spend winter as adults, and most species begin activity early in the spring. After April, females mate and often disperse from the natal nest to search for suitable nesting sites. Females then start to provision brood cells with pollen and a comparatively low proportion of nectar, by weight (2 percent, Maddocks and Palus 1987; 4 percent, E. Strohm

Figure 5.3

ALFALFA LEAFCUTTER BEE EGG LAID ON POLLEN PROVISION MASS IN NEST, MOISTENED WITH NECTAR



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Figure 5.4

ALFALFA LEAFCUTTER BEE EMERGING FROM BROOD CELL

(among many brood cells collected and managed for commercial pollination in Italy)



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unpublished data). Larvae feed, spin a cocoon, pupate, and develop into adults during August to September, and then remain in their cocoons to hibernate (Strohm *et al.* 2002).

Orchard mason bee: Under managed conditions in North America, the dates of bee emergence in the spring are controlled, because the bees are kept in cold storage until transferred to nesting boxes from late-April to mid-June, depending on fruit tree flowering (Sheffield *et al.* 2008b). Males emerge first, followed by females approximately one week later. The female bees carry out nesting activity for about 30 days, and their progeny reach the adult wintering stage by late summer (Rust 1995; Bosch and Kemp 2000, 2001).

Reproduction rate/ ratio of female to male offspring

In most temperate regions, leafcutter bees (some *Megachile* species) have one generation per year (Pinzauti 2000 [Italy]; Asensio 1982 [Spain]; Tasei 1977 [France]; Holm 1983 [Denmark]) although two generations have been observed in both Canada (Krunic 1972; Sheffield *et al.* 2011) and Tuscany, Italy (Pinzauti 2000). In favourable weather conditions female alfalfa bees generally lay about one egg per day during a month. Klostermeyer *et al.* (1973) record around 30 eggs laid per female for the alfalfa bee over a reproductive season.

In Canada, Sheffield *et al.* (2008a,b) recorded recovery rates (the number of bees produced in a nesting site, in relation to the number released) and female fecundity rates (number of offspring per female), in relation to distance from an important forage resource for orchard mason bees. Notably, close proximity to alternative forage, following crop flowering, leads to greatly increased populations in the next season.

However, survival rates and emergence the next spring/summer may be far less than expected. For example, a study of the nesting biology of a native megachilid in Utah, USA, *Megachile pugnata*, indicates that 50 percent of bee offspring fail to reach the adult stage in the following season (Tepedino and Froehlich 1982). For red mason bees, in their study and at other sites, Strohm *et al.* (2002) document mortality rates that range from 19.3 to 43 percent. Sheffield *et al.* (2008a) note rates of pre-emergence mortality ranging from 16.9 to 47.6 percent in managed populations of orchard mason bees.

Nesting resources

Leafcutter bees use diverse nesting sites and nest materials. Making use of many kinds of cavities, they build nests in dead wood, hollow plant stems, rock crevices and other sites such

as cracks in buildings, in snail shells, and even the nostril cavities of a wildebeest skull in Kenya (O'Toole and Raw 1991).

Leafcutter bees are often referred to as “renters” in the sense that they make use of existing cavities for nesting. However, the range of material that they may employ to line, “partition” or plug their brood cells is impressive, including mud, pebbles, leaves, petals, resin and glandular secretions. Here, the focus is on two well-known crop pollinating megachilid bees with different nesting habits. The range of nesting resources and their estimated quantities are presented in Table 5.3.

Alfalfa leafcutter bee

Once the female alfalfa leafcutter bee has received the sperm to fertilize about 30 eggs, she starts to search for a suitable nesting site, i.e. pre-existing natural cavities. Normally she prefers dead stems lying horizontally or nearly so, canes of about 6 mm diameter, or narrow spaces between walls of buildings. After finding a suitable cavity (frequently the same one used by the mother or by a conspecific individual) she begins to gather pieces of fresh leaves to line the nest (Pinzauti 2000).

According to the size of the tunnel, the mother bee cuts from 10 to 30 pieces of leaf with her mandibles to line the cell. After filling cells with pollen and laying an egg on the pollen mass, alfalfa bees will continue to collect leaf pieces and construct a cell partition that separates one brood cell from the next. This occurs for the whole length of the cavity or tunnel, over a maximum length of about 13 to 15 cm. When the last cell in the tunnel is complete, the female will cut many pieces of leaves (up to 50) to finally close the tunnel entrance (Figures 5.7 and 5.8). Under commercial production, a female normally uses two to three nest-tunnels during her life.

The female of red mason bees accepts diverse pre-existing cavities as nest sites (e.g. Westrich 1989). After an egg is glued on the provision mass, the brood cell is sealed with a partition made of more or less loamy soil, which females gather and bring in their mandibles to the nest. Strohm *et al.* (2002) estimate that females need 11.9 ± 4.7 ($n = 55$) trips to gather the loam for a cell partition. One trip lasted 123 ± 45 sec ($n = 56$) and a female brought on average 17.5 ± 5.6 mg ($n = 56$) loam per trip to the nest. Females need 149 ± 48 sec ($n = 56$) to process one load of loam for cell partition construction. A mean mass of 187 ± 51.5 mg loam is used for one cell partition (Strohm *et al.*, 2002). However, the amount of loam gathered per cell partition increases from brood cell to brood cell (Ivanov 2006).



Figure 5.5a and b

ALFALFA LEAFCUTTER BEE NEST “PLUGS” AND EVIDENCE OF LEAF SECTIONS CUT AND USED BY LEAFCUTTER BEES



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Figure 5.6

ALFALFA LEAFCUTTER BEE SEALING THE ENTRANCE TO A NEST TUNNEL



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Table 5.3

NESTING RESOURCE QUANTITIES USED BY ALFALFA BEES AND RED MASON BEES

RESOURCES/QUANTITY USED	ALFALFA LEAFCUTTER BEE (<i>MEGACHILE ROTUNDATA</i>)	RED MASON BEE (<i>OSMIA RUFA</i>)
Nest lining	250 leaf pieces per female	In nest tunnels with large diameters females may line the walls with different amounts of loam (Strohm unpubl. observations)
Nest plug	150 leaf pieces per female	About 250 mg (Ivanov 2006)
Cell partitions		187+51.5 mg per partition 2337 mg of loam, per nest (Strohm <i>et al.</i> 2002) 70-130 mg (Ivanov 2006)
Nest provisions	80% pollen and 20% nectar, to 35% pollen and 64% nectar	217 mg per cell, mostly pollen, 2-4% nectar

DAILY FORAGING PATTERNS

Megachilid bees are prodigious foragers. A female alfalfa leafcutter bee is estimated to have a rate of flower visitation ten times faster than honey bees at alfalfa flowers. (Krunic *et al.* 1995). Typically, 10 to 15 flowers of alfalfa are visited per foraging flight (Pinzauti 2000). Leafcutter bees may forage during cooler and wetter weather than honey bees, as well as both earlier and later in the day (Vicens and Bosch 2000).

Alfalfa leafcutter bee males and females are inactive at temperatures lower than 20°C and reach maximum flight activity at around 30°C. Females normally continue to visit alfalfa flowers even at temperatures of 40°C. Similarly, for red mason bees, nesting activity is considerably reduced on days with a maximum temperature less than 20°C (Strohm *et al.* 2002), but some activity continues to about 15°C (Strohm, unpublished data).

The female red mason bee forages from early morning until dusk. It has been estimated that females need about six and a half hours to provision and seal a brood cell; five hours are spent foraging and storing provisions, with the remainder spent foraging for loam and constructing the cell partition. A female makes on average 18 foraging trips to gather pollen and nectar, each trip lasting 13 minutes. Storage of one provision load takes only about a minute. Twelve foraging trips are needed, on average, to gather the loam for a cell partition. Each trip lasts about two minutes and a female brings 18 mg loam per trip to the nest. An average mass of 187 mg loam is used for one cell partition (Strohm *et al.* 2002).

Pollen collection/consumption

Strohm *et al.* (2002) quantify red mason bee nest provision parameters. Weighing the nests before and after a female had deposited provisions, they quantify the provisions brought to a nest per foraging trip, per brood cell, per day, and throughout the season. One trip results in a mean of 13.5 ± 4.3 mg ($n = 56$) of pollen and nectar. The total average mass of provisions for one brood cell was 217 ± 85 mg ($n = 49$), but size of provision mass differed significantly between male (163 ± 44.2 mg) and female progeny (306 ± 48.9 mg).

Nectar collection/consumption

Strohm *et al.* (2002) note that nectar makes up only a small portion of the provision mass for the red mason bee, from two to four percent, thus it might be assumed that most nectar collected is consumed by an adult bee either male or female.

By contrast, Klostermeyer *et al.* (1973) find that the composition of the provisions differs considerably in the alfalfa leafcutter bee. They find that bees carry provision loads equivalent



to 23 percent of their body weight (average 35 mg), and bring in about 80 percent pollen and 20 percent nectar on the first load of provisions, gradually increasing the amount of nectar in subsequent loads. The final loads are almost entirely nectar, resulting in provisions composed of 65 percent nectar and 35 percent pollen.

Foraging range from the nest

Bees are “central-place foragers”, and Pinzauti (2000) suggests that flight ranges of alfalfa leafcutter bees are short enough that maximum pollination service is realized by placing bee nests no more than 300 m from each other, anticipating a foraging range of 150-200 m. Sheffield *et al.* (2008b) suggest that floral resources at 600 m from an established nest are beyond the foraging range for most female *Osmia lignaria*. Maccagnani *et al.* (2003) find evidence that *Osmia cornuta*, in Italy, forage up to 400 m from its nest.

Crop/host plant preferences

Megachilid bees are somewhat specialized in their forage plant preferences, compared to other important crop pollinators. Many osmiine megachilids depend on a narrow range of plant species for pollen (Westrich, 1989). Fifty-five percent of the Central European osmiine bees are thought to be pollen specialists at the level of plant genus or plant family (Müller *et al.* 1997), preferring flowers of Asteraceae and Fabaceae. Crop-pollinating megachilids will often forage a range of plant species outside of crop bloom, but with some clear preferences. Known megachilid crop pollinators, crops they are known to pollinate, and their distributions are given in Table 5.4. Surprisingly, a large proportion of provisions used by *O. bicornis (rufa)* may consist of oak pollen, a nectarless, wind-pollinated species. In other localities the predominant pollen sources are apple and oilseed rape (Radmacher and Strohm 2010; Strohm unpublished data).

Megachilids are documented as pollinators of cranberries (*Megachile addenda*, *M. rotundata*), coffee (*M. frontalis*), alfalfa, chillies and peppers, soybeans, broad beans, (*M. rotundata*), raspberries, loganberries, blackberries (*Osmia aglaia*, *O. cornuta*), strawberries, (*O. cornuta*), rapeseed, mustard seed, turnip rape, canola (*O. lignaria*), apples (*O. lignaria*, *O. bicornis (rufa)*), apricots, cherries, plums, nectarines, peaches, mirabelle, sloe (*O. lignaria*) and blueberries (*O. lignaria*, *O. ribifloris*). Further details are provided in Table 5.4.

In Nova Scotia, in 2002, Sheffield *et al.* (2008b) record the foraging resources of orchard mason bees during and surrounding apple flowering. Prior to and during the early apple bloom,

the garden yellowrocket (*Barbarea vulgaris*, Brassicaceae) constitutes most (72.3 percent) of the pollen collected at one site, with apple (7.9 percent) and dandelion (*Taraxacum*, Asteraceae – 7.7 percent) contributing smaller amounts. During full to late bloom at this site, 76.1 percent of pollen collected is apple, lupine (11.0 percent), dandelion (2.1 percent) and several unidentified types. At another site, apple accounts for 93.6 percent of collected pollen. Following apple flowering, orchard-associated legumes such as lupine account for 95.6 percent of pollen in one year and 90.7 percent in another.

Table 5.4

MEGACHILID CROP POLLINATORS, DISTRIBUTIONS, AND ASSOCIATIONS

CROP POLLINATOR	CROP POLLINATED	DISTRIBUTION	OTHER ASSOCIATIONS
<i>Megachile addenda</i>, a leafcutter bee	Cranberries (<i>Vaccinium macrocarpon</i> , <i>V. oxycoccus</i>)	North America	A pollinator of <i>Diervilla lonicera</i> (Caprifoliaceae); <i>Psoralea onobrychis</i> ; groundnut, <i>Arachis hypogaea</i> (Fabaceae)
<i>Megachile frontalis</i>, a leafcutter bee	Coffee, arabica (<i>Coffea arabica</i>); coffee, robusta (<i>C. canephora</i>)	Southeast Asia and Australasia	Forages a wide range of forest and forest-edge flowers
<i>Megachile rotundata</i>, an alfalfa bee	Alfalfa (<i>Medicago sativa</i>); chiles and peppers (<i>Capsicum annuum</i> var. <i>annuum</i> ; <i>C. frutescens</i>); cranberries (<i>Vaccinium macrocarpon</i> , <i>V. oxycoccus</i>); soybeans (<i>Glycine max</i>); broad beans (<i>Vicia faba</i>)	Europe and North Africa, eastwards to the Caucasus, central and eastern Asia. Introduced to North and South America and New Zealand.	A polylectic species, with a preference for Asteraceae and Fabaceae pollen; in the US almost specializing on alfalfa blossoms.
<i>Osmia aglaia</i>, a mason bee	Raspberries (<i>Rubus idaeus</i>); loganberry (<i>R. loganobaccus</i>); blackberry (<i>R. plicatus</i>)	North America	Not known
<i>Osmia cornuta</i>, European orchard bee	Strawberries (<i>Fragaria × ananassa</i>); almonds (<i>Prunus dulcis</i>); raspberries (<i>Rubus idaeus</i>); loganberry (<i>R. loganobaccus</i>); blackberry (<i>R. plicatus</i>)	Widespread in south, central and eastern Europe, with the range extending eastwards into central Asia, north Africa and the Levant. It is adventive in North America.	A broadly polylectic species, known to forage on Asteraceae, Fabaceae, Rosaceae including apples, prunes and pears. Maccagnani <i>et al.</i> (2002), however, found only pollen of <i>Pyrus</i> in nests of <i>Osmia cornuta</i> near pear orchards.
<i>Osmia lignaria</i>, Blue orchard bee	Rapeseed; oilseed rape (<i>Brassica napus napus</i>); mustard seed (<i>B. nigra</i> , <i>B. juncea</i> , <i>Sinapis alba alba</i>); turnip rape; canola (<i>B. rapa rapa</i>); apples (<i>Malus domestica</i>); apricots (<i>P. armeniaca</i>); cherries (<i>P. avium</i> , <i>P. cerasus</i>); plums (<i>P. domestica domestica</i>); nectarines (<i>P. persica nucipersica</i>); peaches (<i>P. persica persica</i>); greengage; mirabelle; sloe (<i>Prunus spinosa</i>); blueberries (<i>Vaccinium angustifolium</i> , <i>V. corymbosum</i> , <i>V. pallidum</i>); bilberry (<i>V. myrtillus</i>)	North America	A broadly polylectic species, known to forage on Fabaceae, Rosaceae among others.



CONCLUSIONS

Natural history information on seasonal patterns of megachilids visiting crops has thus far been recorded in North America and Europe, although megachilid bees are abundant elsewhere and in tropical zones. There is a lack of understanding of their seasonal patterns under many conditions. The same is largely true for reproductive rates and foraging patterns with respect to pollen, nectar and nesting material.

From what is known regarding well studied Northern Hemisphere species of leafcutter bees, they may be particularly exposed to pesticides when gathering a wide range of material in agricultural environments. Their foraging time throughout a day is probably longer than that of honey bees, and they may be more tolerant of cold conditions. Being more specialized than honey bees on particular plant species, it appears that females will concentrate on gathering pollen from massively flowering but short-blooming species such as apple, pear and oilseed rape, so that any pesticides applied to these crops during the bloom will surely be found in the bees' pollen masses. The active life span of osmiine bees is not much longer than the fruit tree bloom. The pesticides applied during the bloom period will affect them heavily at the peak of their activity.

Contaminated soil or leaves may potentially impact these bees as much as pesticides landing on flowers. Clearly the basic good practice of avoiding pesticide risk to pollinators applies well to megachilid bees: no pesticide application during bloom, and avoidance of agrochemicals that are particularly toxic to bees. As already mentioned, the need to avoid drift and spray of biocides on non-targets is also critical for megachilids. Equally, use of pesticides in gardens and for landscaping purposes may affect the leafcutter and mason bees when they have prolonged contact with leaves gathered for nesting purposes. The value of available floral resources, before and after crop blooms, to produce healthy, abundant bees is evident, e.g. from studies on orchard mason bees in Canada. Such practices might offset losses due to agrochemicals.



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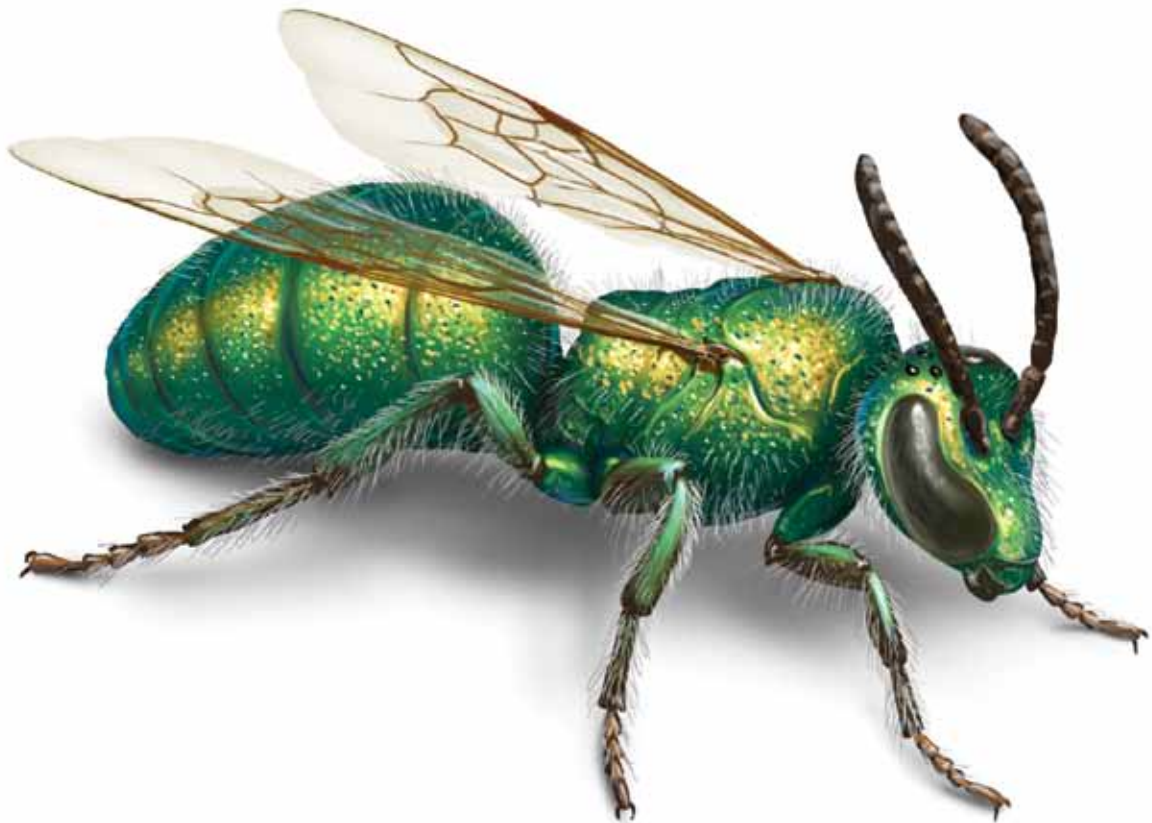
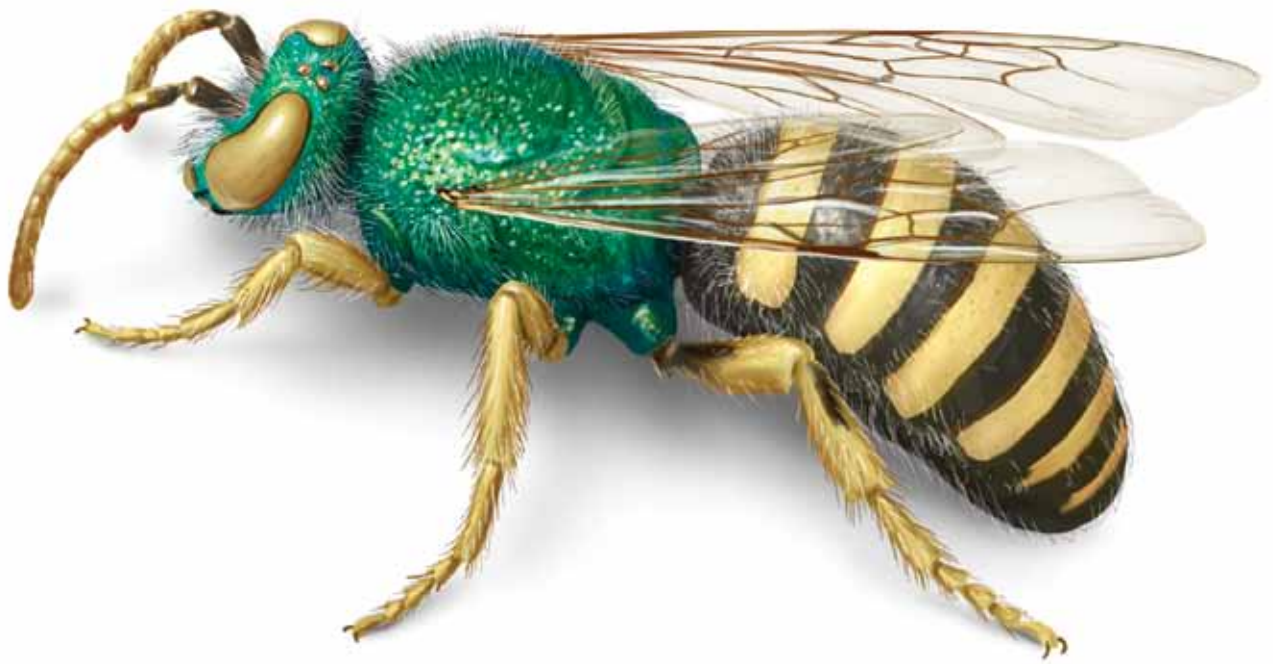


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Pollinator-dependent crops: kiwi, above; onion (seed), below.

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Top: Agopostemon; bottom: Augochorella.

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CHAPTER 6

SWEAT BEES (HALICTIDAE): NATURAL HISTORY AND PESTICIDE EXPOSURE

Dino J. Martins

INTRODUCTION

Halictidae are a diverse and widespread family, found in all terrestrial biogeographic regions of the world. They are known as “sweat bees” from the habit of seeking salts from perspiration on humans and domestic animals, though this behaviour is not limited to halictids and found in several other bees including members of the Megachilidae and Apidae. In some temperate habitats halictids may nearly dominate the bee fauna, less abundant only than the honey bee (Michener 2000).

There are over 4 000 described species of these “short-tongued” bees¹. Halictids are small to medium-sized (Table 6.1), many with metallic blue or green coloration, often in bands across the abdomen. Many also are black and brown and often have metallic hues, or bands of pale hairs. The four subfamilies of Halictidae are Rophitinae, Nomiinae, Halictinae and Nomoidinae (Michener 2000; Borror *et al.* 1989).

1 http://www.discoverlife.org/mp/20q?guide=Apoidea_species&flags=HAS



Table 6.1

AVERAGE BODY MASS (LIVE WEIGHT) OF HALICTIDAE

SPECIES	COUNTRY	AVERAGE BODY WEIGHT	REFERENCE
<i>Nomia melanderi</i> 'alkali bee'	USA	Males: 96.8 mg (15.8 s.d.) Females: 83.0 mg (14.4 s.d.)	(Rust 2006)
<i>Nomioides variegatus</i>	France	Male: 2.56 mg Female: 2.80 mg (note that these are dry weights)	(Rust <i>et al.</i> 2004)
<i>Halictus rubicundus</i>	The Netherlands	Male pupae: 0.063 g (0.003 s.d.) Female pupae: 0.082 g (0.0006 s.d.)	(Hogendoorn and Leys 1997)
<i>H. ligatus</i>	Canada	Males: 6.59 mg (1.08 s.d.) Females: 5.64 mg (1.14 s.d.) (note that these are dry weights)	(Richards and Packer 1994)

NATURAL HISTORY OF HALICTIDAE IN RELATION TO FORAGING AND POLLINATION

Given the high diversity within Halictidae, it is not surprising that their foraging habits are varied. Most are not well studied, but among species that are better researched is the alkali bee (*Nomia melanderi*). It has been recorded visiting flowers in 48 genera within 21 families, with a predominance of Fabaceae and Asteraceae (Rust 2006). Although they are important pollinators for some crops and are managed for alfalfa pollination (Cane 2008), halictids depend strongly on native flowers and can be fairly selective, or even restricted to certain small plant groups.

Halictids appear to be most active in flight (during sunny periods), as are many bees (Martins 2003; Klein *et al.* 2007; Williams *et al.* 2010, 2011). In some species the males appear to visit flowers later in the day and sometimes spend the night in the flowers (pers. obs.). This needs to be taken into consideration in assessing pesticide exposure. Furthermore, some halictids are nocturnal or crepuscular. Because pesticide application regimes often focus on those periods to avoid poisoning the diurnal bees, this part of halictid biology should be seriously considered, especially in subtropical and tropical habitats.

Halictids are highly seasonal (Rust 2006; Schwarz *et al.* 2007). Many species are adapted to synchronized seasonal emergence or sharp population peaks, followed by intensive foraging, and provisioning of brood cells. Their limited flight periods and foraging patterns make them especially vulnerable to pesticide exposure in both temperate and tropical ecosystems, if, of course, pesticide exposure coincides with their activity. In seasonal tropical dryland ecosystems that experience distinct wet and dry seasons, many solitary bee species have synchronised adult

emergence and foraging patterns (Roubik 1989; Martins 2003). At such times, even a single major exposure during the foraging and brood provisioning period can be devastating. Better data on these life history patterns, in different environments, would inform pesticide application calendars and additional human factors that impact wild bees (Winfree *et al.* 2009).

There are not much data available on the number of days per season in which halictids forage. However, alkali bee research suggests a wide range of foraging patterns in different environments. Bees in warmer environments may forage over a longer time period, while those in more temperate areas are more restricted and therefore potentially more vulnerable — when pesticide application coincides with their foraging. Studies suggest large differences in the number of days halictids spend foraging in a season, even in a relatively uniform climatic period. For example, in a study of *Halictus rubicundus* in the Netherlands, those halictids were found to be a single generation (univoltine) in cooler areas and bivoltine (two broods a year) at more sheltered sites (Hogendoorn and Leys 1997). The two bee populations were only 150 km apart. Similar patterns are observed in studies of *Halictus rubicundus* in New York, USA, where variation in the number of broods and environmental effects on male production are documented by Yanega (1988-1993).

There are some data that demonstrate the effects of pesticides on the alkali bee. It is the only species of halictid for which controlled, tested pesticide exposure information is available (Johansen and Eves 1963; Torchio 1973). In standardized LD₅₀ trials the alkali bee was tested with DDT, toxaphene and parathion. This halictid is the most susceptible species to these three chemicals (the other two species being instead tested were honey bees and the alfalfa leafcutter bee, *Megachile rotundata*). The alkali bee is, however, less susceptible to phosmamidion, dimethoate and malathion (Torchio 1973). The LD₅₀ data from the study are presented in Table 6.2.

Halictids have been widely observed to include species that are oligolectic (Figures 6.1 and 6.3). They specialize on pollen from a limited number of plant families and even just a few species. For example, *Systropha* feed primarily on Convolvulaceae while a *Xeralictus* appears oligolectic on *Mentzelia* (Loasaceae), found in southwestern USA (Michener 2000).

In the dry areas of eastern Africa, halictids have been widely recorded as abundant and frequent flower visitors. Some are only observed on a few species of flowers, while others are consummate generalists that visit many of the available flower species. *Lasioglossum* are common visitors and pollinators of *Barleria* and other Acanthaceae. *Pseudapis* are widely seen visiting the flowers of *Aloe* to collect pollen during the protandrous (pollen-only) floral phase, and are an important pollinator of *Indigofera* (D. Martins, unpublished data). *Nomioides* can seasonally be among the most abundant bee visitors to wildflowers in France (Rust *et al.* 2004);



Table 6.2

EXPERIMENTAL LD₅₀ SCORES FOR THE HALICTID BEE, *NOMIA MELANDERI*

PESTICIDE TESTED	LD ₅₀ OBSERVED IN <i>NOMIA MELANDERI</i>
DDT (93%)	0.0074
Toxaphene (71%)	0.0023
Mevinphos (75%)	0.0022
Trichlorfon (45.3%)	0.0465
Oxydementonmethyl (50%)	0.0082
Demeton (99%)	0.0260
Tepp (100%)	0.0032
Naled (64.5%)	0.0016
Parathion (95%)	0.0015
Diazinon (48%)	0.0020
Dieldrin (17.9%)	0.0023
Dimethoate (46%)	0.0021
Malathion (57%)	0.0036
Phosphamidon (80%)	0.0054
Dicrotophos (90%)	0.0010

Source: Torchio, 1973

in East Africa they visit *Acacia* (Martins 2003) and are found on *Heliotropium zeylanicum*, *Tribulus* and *Argemone mexicana* (pers. obs.). *Nomia* (Subgenus *Lipotriches*) has been recorded on many different plants, including *Acacia* in East Africa, and flowering grasses and sedges (Bogdan 1962; Immelman and Eardley 2000; Gemmill and Martins 2003).

Many halictids opportunistically forage on invasive and weedy species like *Argemone* that may be targets for control or removal/eradication. The management of such weedy species with herbicides needs to be carefully evaluated, especially in environments where they are a significant proportion of the alternative nectar resources available to halictids.

The main crops of interest where halictids have been studied or managed are alfalfa (*Medicago sativa*) and to a lesser extent vegetables managed for seed (Baird *et al.* 1991), such as onion (*Allium cepa*). *Macronomia rufipes* is a specialized pollinator on eggplant (*Solanum melongena*) in East Africa (Gemmill-Herren and Ochieng 2008). *Lipotriches* and *Halictus* are also common buzz pollinators (bees that vibrate their flight muscles to sonicate anthers and thereby release pollen from a pore at the anther tip) on eggplant and other members of the Solanaceae in East Africa. Halictids contribute to the pollination of watermelon (*Citrullus lanatus*) both in the USA and East Africa (Kremen *et al.* 2002; Njoroge *et al.* 2004). *Halictus tripartatus* pollinates watermelon in the western USA where it is also a pollinator of prickly pear (*Opuntia*; Parfitt 1980). *Lasioglossum* has

Figure 6.1

HALICTIDAE FORAGING IN KENYA



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(a) *Nomia* sp. on flowers of eggplant (*Solanum melongena*), Baringo, Kenya; (b) *Lipotriches* sp. approaching a flower of *Solanum incanum*, Laikipia, Kenya; (c) *Halictus* (*Seladonia*) sp. on Asteraceae flowers, Laikipia, Kenya; and (d) *Systropha* sp. visiting a flower of *Ipomea*, Mogotio, North Rift, Kenya



been recorded on sunflower (*Helianthus annuus*) and apple (*Malus*). The mining bees *Homalictus* are pollinators of Macadamia nuts (*Macadamia ternifolia*; Free 1993). Halictids are also known to contribute to blueberry pollination (Isaacs and Kirk 2010).

The foraging ranges of halictid species should be correlated with their relatively small size (Greenleaf *et al.* 2007), but remain unknown. From observations on farming and dryland systems in Eastern Africa, it appears that many tropical dryland halictid species have fairly restricted foraging ranges (Martins 2003). On small scale or subsistence farms, often rich in halictid species, pesticide exposure needs to be limited through understanding and managing bees more directly than in larger-scale more commercially developed farming systems, because small-scale farms often have more of an 'edge' in terms of being adjacent to natural habitat or fallow areas where halictids are likely to be nesting/foraging. Many halictids also spend extensive periods resting on foliage between foraging bouts. This, too, is important to consider in assessing pesticide exposure risk, since foliage may retain residues or metabolites that could be harmful to bees.

NATURAL HISTORY OF HALICTIDS IN RELATION TO BROOD CARE

An important aspect of halictid biology for assessing the risks of pesticide exposure is the diverse sociality in this family (Packer *et al.* 2007). The Halictidae include solitary species that can nest either alone (dispersed), or in aggregations. There are also some social species, including small colonies that are considered parasocial, subsocial and quasisocial and persist up to several months. The degree of sociality in some groups appears to be influenced strongly by environmental conditions and this is of particular relevance in agroecosystems where such environmental conditions are modified or extended by various farming practices (Borror *et al.* 1989; Roubik, 1989, 2012). For example, modification by agricultural practices could take the form of creating areas of bare ground with greater sun exposure lead to higher success for social species that develop nest aggregations.

Eusocial species of halictids have perennial colonies lasting 4 to 5 years. These communal nesters have workers that share responsibility for rearing offspring (sisters) with a gyne (female reproductive) laying eggs that develop into new workers, males and future queens.

One general trend observed of halictids is that eusocial species are more widespread and successful. For example, *Halictus ligatus* and *Lasioglossum malachurum* are two strictly eusocial species in temperate Europe and North America. There they are among the most successful of bees, when measured in terms of both abundance and diversity of habitats occupied (Michener 2000). They have reduced breeding seasons in a 'delayed eusociality' system where queens

and workers overwinter, then emerge in the following spring to produce brood. Queens and workers thus emerge early in the season as mature adults and begin to forage. This extends their potential exposure to many pesticides (Schwarz *et al.* 2007), and their food resources need to be free of potential contamination across seasons.

Halictid diversity, and the difference in life history between solitary and social species, remain to be studied in greater detail, in order to better understand pesticide exposure risks.

NATURAL HISTORY OF HALICTIDS IN RELATION TO NESTING RESOURCES AND REPRODUCTION

Nesting patterns and nest-site choice are of particular relevance for Halictidae, in relation to their pesticide exposure. Halictid species typically nest in burrows, either in soil or in dead wood. Earth banks, sheltered rocks and bare or level ground can often hold aggregations of nests, and these appear to be used by sequential bee generations (Roubik 1989; Michener 2000). Large nest aggregations correspond to specific soil moisture, pH and drainage conditions. Those nesting aggregations are of special concern. Localized sub-populations at these sites may number in the thousands, and exposure could impact a large portion of those pollinators (Figure 6.2).

Alkali bees are managed using artificial nesting sites to enhance alfalfa pollination (Torchio 1973; Wilchens *et al.* 1992; Rust 2006). Every attempt needs to be made to prevent pesticide run-offs into such halictid nest aggregations.

There are major gaps in basic life history information for many bees. The Halictidae span a wide range of social behavioral strategies (Sakagami and Michener 1962). They include variation

Figure 6.2

**GROUND NESTING SITE OF SOLITARY BEES (*ANDRENA VARGA*)
AGGREGATED IN A SMALL AREA**



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Figure 6.3

SMALL SOLITARY BEES IN KENYA



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(a) *Lipotriches* sp. resting on a leaf of *Solanum incanum* in between foraging, Laikipia, Kenya; (b) *Nomioides* sp. on a flower of *Tribulus terrestris*, South Turkwel, Turkana, Kenya; (c) Long-faced bee, *Thrincostoma* sp. resting on a leaf at forest edge, Kakamega Forest, Kenya; and (d) Long-faced bee, *Thrincostoma* sp. foraging on a flower of *Justicia flava*, Kakamega Forest, Kenya

in the number of individuals housed in a nest as well as variation in division of labor in castes. This is further embellished by both interspecific and intraspecific variability (Richards 2000; Soucy and Danforth 2002; Michener 1974; Wcislo *et al.* 1993). Studies continue to uncover examples of sociality in halictids. For instance, communal nesting in the South African *Patellapis*, a diverse genus, was recently described (Timmerman and Kuhlmann 2008).

The sharing of nests, the sometimes large nest aggregations, and sharing of food between nest-mates all raise the issue of potentially multiplying the effect of a single foragers' exposure to pesticides — to multiple individuals. The implications for sociality and exposure in halictids are outlined below.

SOCIALITY, BROOD CARE AND GREGARIOUS BEHAVIOR, AND IMPLICATIONS FOR EXPOSURE IN HALICTIDS

- Single foragers return to nests where they may share nectar with multiple brood or nest mates through regurgitation (trophallaxis). They may also share pollen food, which potentially extends exposure of one individual to many.
- Shared food resources in a single nest aggregation or single nest potentially concentrate residues. Concentration levels that might be below those considered detrimental in the environment, or in crop fields, may be augmented within the nest, due to storage of both pollen and nectar (Richards and Packer 1994).
- Foragers may also share nest-building materials, even where actual brood food resources are not shared. Glandular secretions or gathered materials for construction of, for example, the lining of the nest tunnel walls, may be exposed and thus extend exposure to other individuals.
- Aggregated nests at the edges of fields are directly vulnerable, with serious consequences for exposure at a local population level. For example, bees in East Africa nest alongside 'bomas' (traditional livestock enclosures), often sprayed or treated for ticks, biting flies, etc.
- Sociality typically goes hand-in-hand with multivoltine (multiple generations per season or year) life history and this expands both active season and total exposure.
- Sociality influences the volume of pollen consumed by different kinds of brood. Some halictids have caste-variation in larval size and development. Typically the largest larvae become reproductive individuals. Therefore, exposure to even small amounts of residues has the potential to affect the next generation and number of reproducing individuals, although this needs more study (Richards and Packer 1994; Hogendoorn and Leys 1997).



CONCLUSIONS

There is a deficiency in the information on both the direct and the multiplied or 'downstream' effects of exposure to pesticides among halictids, which remains to be addressed. Management techniques used for honey bees could be extended for use with halictids, but halictid sociality is more varied, and nest sites and aggregations may be unrecognized within agroecological landscapes. In small-scale intensive farming, farmers could use the following basic questions as a guide to managing their pesticide use, so as limit the potential exposure of halictid bees:

- What are the seasonal patterns of bee foraging in relation to the crop calendar? A calendar of crop phenology and spraying regimes needs to be developed alongside that of bee abundance, seasonality, flower visitation and nesting pattern.
- Where in the landscape are nest aggregations and other resources relevant to both solitary and social species? Limiting or preventing exposure at these sites is a key component for protecting the bees.
- What happens to residues on leaves, drainage ditches and in soils? Potential ways of mitigating such exposure is through careful spraying regimes, strict adherence to manufacturer user guidelines and working with extension agents and agro-chemical suppliers for up-to-date information.

Critical gaps in knowledge about halictids:

- There are no measurements or direct data on the toxicity of pesticides to halictids, for most if not all widely-used chemicals and their formulations. Work with known crop pollinators can be matched with studies of wild halictids under different pesticide exposure regimes.
- More detailed studies are needed of aggregated/communal nest sites. For example, what factors guide bees to select certain sites? Information on such criteria will enable farmers to manage landscapes for halictids more effectively.
- More information is needed on floral calendars and flower species used by halictid bees when foraging away from crop fields.
- More study is also needed on details of the general biology and foraging patterns of tropical halictids, both in wet and dry environments.

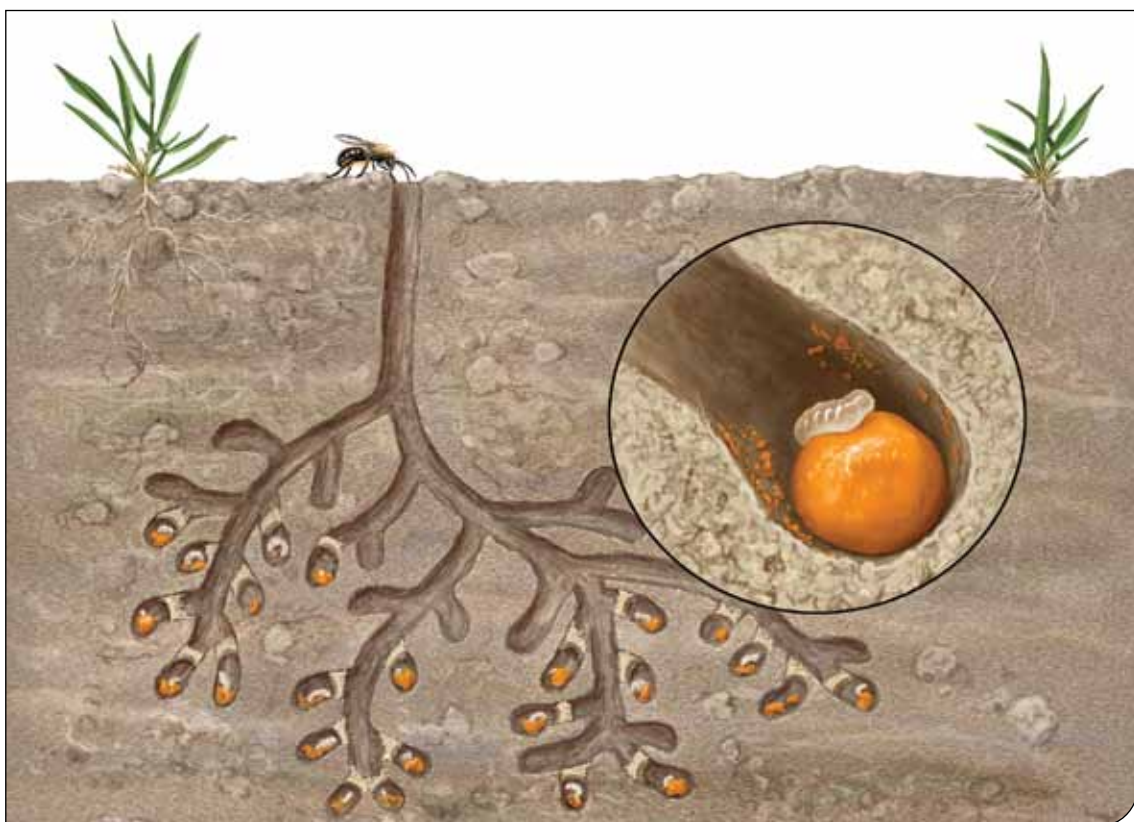
Management practices that can reduce risks to halictids:

- Identify halictids as a component of wild bee fauna on crops and as important pollinators.
- Avoid spraying crop field edges, compacted earth sites, sheltered banks.
- Map and protect aggregated/communal nest sites.

- Construct and protect artificial nest sites for communal species.
- Develop spraying regimes that avoid critical foraging periods to limit direct exposure of adults to toxins — in particular avoid spraying flowers. Take into consideration exposure routes, through direct action of active ingredients as well as through secondary metabolites.

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Underground features of the nest of a mining bee (e.g. Diadasia or Mellisodes). Cells show larvae feeding upon bright orange pollen masses.



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Top: carpenter robbing; bottom: carpenter bee nest.

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CHAPTER 7

ASSESSMENT OF LARGE BEE (*XYLOCOPA* AND *AMEGILLA*) EXPOSURE TO PESTICIDES

Mary W. Gikungu

INTRODUCTION

The large, communal and social carpenter bees of the genus *Xylocopa* (Xylocopinae: Xylocopini) and the solitary bees of the genus *Amegilla* (Apinae: Anthophorini) are extremely active and noticeable bees on farms. They both are capable of buzz pollinating—a mechanism involving vibration of flowers—to obtain pollen, making them pollinators of crops requiring this mechanism, including tomatoes and chilli peppers. Both bees are important in crop pollination, particularly in tropical areas as the production of horticultural crops increases.

Most investigation of pesticide impact on pollinators comes from studies using honey bees, *Apis mellifera*. There is thus considerable information on the foraging behavior of social bees and their risk of exposure to pesticides (Rortais *et al.* 2005; Desneux *et al.* 2007). In contrast, studies on solitary bee exposure to pesticides are few, despite a growing awareness of pesticide impact that affects them (Brittain *et al.* 2010; Williams *et al.* 2010). As a general rule, it has been suggested that solitary bees may be more vulnerable to chemical exposure due to their small body size, short foraging range (less than 500 m for the majority) and plant resource specialization. However, *Xylocopa*, which has small colonies of a female and her brood, and *Amegilla*, among other bees, are quite large; thus the general rules for smaller or less social bees often do not apply well to them. The possible pesticide exposure risks of these larger bees merit careful consideration, some of which is supplied here.



There are indications that larger bees (with relatively small surface area to volume ratios) are less vulnerable to chemical exposure than smaller bees (Fischer and Moriarty 2011). This insight is gained from their considerably larger mass compared to their 'exposed' surface area. The expectation is that their direct contact, and even ingestion of pesticides would have better chances of detoxification, given greater capacity inherent in larger body size. However, this postulated mechanism may be only true for a particular exposure incidence.

Larger bees generally live for a few months or more, thereby outliving an individual crop or wild plant blooming period, and also many other kinds of bees, including honey bee workers. In addition, they may fly large distances in search of floral resources and nesting sites. In this way, the total exposure for an individual bee, over time and space may be greater than for smaller bees, and exposure to pesticides may be relatively great. However, large bees forage over comparatively larger areas than smaller bees, and this may actually result in diluting their exposure to food or areas contaminated with chemicals and thus reducing their exposure compared to smaller bees.

FORAGING, NESTING AND PROVISIONING BEHAVIOR OF *XYLOCOPA*

Seasonality and life cycles of *Xylocopa*

There are about 400 species of *Xylocopa* (large carpenter bees) which inhabit a broad range of ecosystems in the tropics, subtropics and temperate regions of the world. Carpenter bees live up to three years, and have the potential for multiple annual generations and maintaining small colonies. When brood emerge those adults frequently stay within the parental nest, and females sometimes mate and then initiate their own tunnels in the same branch or tree trunk. Because they make tunnels in non-living wood with a fairly low moisture content, they are often residents of commercial timber and often, large buildings. The number of bee generations per year varies with climatic factors, from one to around four (Bonelli 1976). The same female can produce successive broods in one season (Gerling *et al.* 1989).

There are distinct aspects of *Xylocopa* life cycles that may lead to predictions of vulnerability to pesticide exposure. Specifically, there may be a considerable amount of food sharing within a nest, through rudimentary sociality. In nesting cycles, there is an extended period of reproductive quiescence in which brothers and sisters stay in the nest; this may be during the dry season in the tropics, or in cooler months in temperate regions (Gerling *et al.* 1989), and may last up to eight months. Young males of some species may solicit, and be fed, regurgitated nectar from their sisters, for example in *X. caffra* in Africa (O'Toole and Raw 1999). Among *X. pubescens* in

Israel, it has been noted that when there is more than one generation in a year, some of the daughters remain in the nest. They will guard the nest while the mother forages, but upon her return they may force her to feed them nectar, through a process called trophallaxis (O'Toole and Raw 1999). Bees that guard nests, and young adults, may also feed on pollen brought to the nest to provision brood cells (Gerling *et al.* 1989). In this way, contaminants brought to the nest by some foragers may be shared within a colony.

Most carpenter bees have two to four brood cycles per year. For example, Ethiopian *X. combusta* shows up to four yearly brood cycles corresponding to two generations (Bonelli, 1976). Beeson (1938) also made similar observations of *X. latipes*. Around Lake Victoria in East Africa Anzenberger (1977) found that *X. nigrita*, *X. flavorufa*, and *X. caffra* have two broods per year.

In general, the life span of an adult bee is terminated when her brood emerge as adults (Roubik 1989), but the emerging adults of *Xylocopa* may remain in the parental nest and assist long-lived mothers, and siblings, to provision cells.

Foraging characteristics

Xylocopines may be long-lived not just as adults, but also as immatures that have a two- to three-year lifespan. Roubik (1989) suggests that with their long life spans and slow metabolic rates during adult diapause, they are able to endure dearth periods of low resource availability. When flowers do become available in large number as in the general flowering period in the Southeast Asian dipterocarp forests, they appear able to rapidly build up population levels. Bursts in reproductive and foraging activity may consequently shorten the life spans of the population, with the result that mortality may be higher during and just after mass blooming periods. Whether there are population peaks at times of mass flowering crops is not established in the literature.

The flight ranges of xylocopines are amongst the greatest of all bees. Female *Xylocopa* have been released at distances from 5 to 12 kilometers from their nests and have successfully returned home (Balduf 1962; Kapil and Dhaliwal 1969); the latter authors estimate that Indian species of *Xylocopa* may fly as far as 20 km. Recently, the flight ranges of female *Xylocopa* in Kenya were measured directly up to 6 km, from bees fitted with tiny radio transmitters (Pasquet *et al.* 2008). With such large flight ranges, most foraging is unlikely to take place close to nests, particularly where there are nesting aggregations. Roubik (1989) estimates that for bees with a maximum flight range of 20 km, peak foraging could be expected to occur between 6.7 and 10 km from the nest.



With long life spans and wide foraging ranges, *Xylocopa* are typically generalists (polylectic), feeding from a great diversity of plant species (Figure 7.1a and b). A carpenter bee in the Galapagos Islands (where there are no other bees to compete with) was recorded visiting 160 species in 28 different plant families (Linsley *et al.* 1966). Most species are diurnal, but the subgenus *Nyctomelitta* has species that forage at night, and some xylocopines forage mainly at dusk. They may visit a broad range of flowers even within a single a day. For example, *Xylocopa gualeanensis* in Costa Rica was observed foraging on at least three different plant species, with peaks at different times, from sunrise to 4 p.m. (Sage 1968). Another species, *X. frontalis*, found in Central and South America has been recorded on the following plant species: *Helianthus annuus* (Asteraceae); *Tabebuia chrysotricha* (Bignoniaceae); *Senna macranthera* (Caesalpiaceae); *Canavalia paraguariensis*; *Phaseolus vulgaris* (Fabaceae); *Sinningia macrostachya* (Gesneriaceae); *Leonurus sibiricus* (Lamiaceae); *Tibouchina gracilis* (Melastomataceae); *Melia azedarach* (Meliaceae); *Passiflora caerulea* (Passifloraceae); *Serjania meridionalis* (Sapindaceae); *Styrax leprosus* (Styracaceae); *Aloysia gratissima*; and *Stachytarpheta cayennensis* (Verbenaceae) (Schlindwein 2003).

As noted above, *Xylocopa* are widely documented for their role in crop pollination systems, especially in the tropics. *Xylocopa aestuans* is recorded foraging from the sword bean, horse bean and other *Canavalia* in Southeast Asia (Gross 1993). *Xylocopa dejeanii* and *X. aestuans* are recorded on coffee (*Coffea canephora*) as part of a suite of wild bee species providing pollination services in the Indo-Malayan region (Klein *et al.* 2003). Studies in Brazil record *X. frontalis* and *X. suspecta* pollinating passion fruit (*Passiflora edulis*) (Schlindwein 2003). In the West Indies *Xylocopa mordax* is the major pollinator of yellow passionfruit (*Passiflora edulis flavicarpa*) (Corbet and Wilmer 1980). In East Africa *Xylocopa* forage from pigeon peas (*Cajanus cajan*), where they are one of the most abundant visitors (Martins 2006) and they also play an important role in the pollination of cowpea (*Vigna*) in the region (Pasquet *et al.* 2008), eggplant (*Solanum melongena*) (Gemmell-Herren and Ochieng 2008) and watermelon (*Citrullus lanatus*) (Njoroge *et al.* 2004). In addition, in tropical Asia they are pollinators of the Neotropical spice plant, *Bixa orellana* (Bixaceae) (D. W. Roubik, pers. comm.).

Floral resources and weather are the key determinants of bee foraging behavior. In tropical and subtropical ecosystems most *Xylocopa* are active throughout the year, although levels of activity are constrained by weather conditions on any particular day. According to Pasquet *et al.* (2008), foraging distances of *Xylocopa* become shorter than the maximum flight range during adverse weather conditions. Even where floral resources are available bees are found to fly for short distances in poor weather conditions (Roubik 1989).

Figure 7.1a

XYLOCOPA INCONSTANS FORAGING ON A PIGEON PEA (*CAJANUS CAJAN*) IN TANZANIA



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Figure 7.1b

XYLOCOPA SP. FORAGING ON *LAGENARIA SICERARIA* AT THE EDGE OF A FARM IN KAJIADO, KENYA



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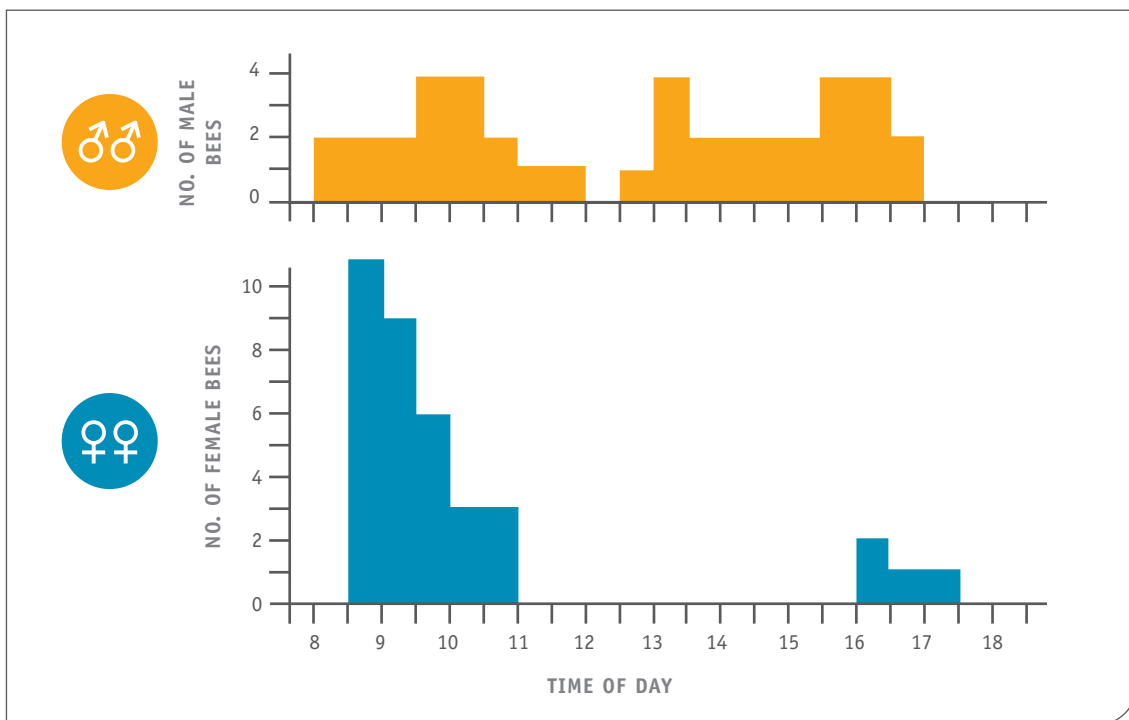
As mentioned, many *Xylocopa* forage long hours throughout a day, including at dusk and dawn in tropical areas (Roubik 1989) and some species have even been recorded foraging and pollinating at night. *Heterophragma quadriculare* is a self-incompatible tree pollinated at night by the carpenter bee *Xylocopa* (*Mesotrichia*) *tenuiscapa* in India (Somanathan and Borges 2001).

Foraging activity shows a distinct pattern, often peaking in the morning and late afternoon (Figure 7.2). Large carpenter bees do not generally forage at temperatures below 12°C, but do appear able to remain active, hovering and foraging at high temperature between 35-40°C. Xylocopines have mechanisms both to dissipate heat generated by their flight muscles to their abdomen—thus permitting them to withstand often high temperatures—and to carry out pre-flight warm-up movements (Heinrich and Buchmann 1986). In the latter case, a hairless and otherwise poorly insulated *Xylocopa* was capable, through such movements, to raise its body temperatures by 8°C above ambient temperature, permitting early foraging on cool mornings.

A single nectar and pollen foraging trip by *X. tranquebarorum*, a nocturnal bee, takes about 17 minutes, and 20 to 40 trips are required to provision one cell (Chun Ling *et al.* 2009).

Figure 7.2

OCCURRENCE OF *XYLOCOPA TORRIDA* ON *CASSIA ABBREVIATA*; N = NO. OF ANIMALS; T = TIME OF DAY (REDRAWN FROM ANZENBERGER 1977)



Similarly, Anzenberger (1977) estimates collecting trips last for 15-25 minutes in a diurnal *Xylocopa*, followed by 15 minutes in the nest before flying out again. Collecting patterns seem to be constrained by the need to guard nests from invaders, as exposed pollen in the nest is attractive to many other species such as ants, which will often need to be chased out when a foraging bee returns. Carpenter bees that use nest entrance repellents (secretions produced by the bees themselves) have been observed to forage longer when compared to bees that do not have that ability to ward off nest predators. Use of chemical repellents (made by the nest foundress) thus has direct influence on foraging duration, and those *Xylocopa* can spend more than 25 minutes foraging (Kapil and Dhaliwal 1968).

Male *Xylocopa* may delay their daily time of feeding at flowers so that morning hours can be spent pursuing females as they forage (Louw and Nicholson 1983). The male *Xylocopa* of certain species, such as *X. virginica*, are often found congregating around patches with rich floral resources, likely to be visited by females (Baird 1986; Barthell and Baird 2004).

Provisioning rates

Carpenter bees vary in size from about 12 to 30 mm in length, and are thus medium to large adult bees. Average body weights drawn from the literature are given in Table 7.1. Foraging distances and trip duration have a direct and positive correlation to bee body size (Gathmann and Tscharntke 2002). Because larger bees have the ability to fly longer distances (Gathmann *et al.* 1994), far beyond their nests, this may increase their vulnerability to pesticide exposure. According to Neff (2008) the amount of resource collected per hour also has a direct correlation with forager body weight. This implies that larger bees collect nectar and pollen loads at a far greater rate, compared to small bees, which may correlate to greater exposure in a given time, when foraging on crops exposed to pesticides.

Table 7.1

SUMMARY OF AVERAGE BODY WEIGHT AMONG *XYLOCOPA*

SPECIES	COUNTRY	AVERAGE BODY WEIGHT	REFERENCE
<i>Xylocopa virginica</i>	USA	Females: 60.3 mg Males: 57.0 mg	Gerling and Herman 1978
<i>Xylocopa virginica</i>	Canada (Ontario) - USA (Maryland)	Females: 58.13 mg	Skandalis <i>et al.</i> 2009
<i>Xylocopa californica</i>	USA (California)	34 to 110 mg	Chappell 1982



Carpenter bees both collect nectar and pollen through handling a flower conventionally, and by “nectar robbing”—creating a slit in the corolla tube or wall of the calyx to extract nectar, without interacting with stamens or pistils. *Xylocopa*, as large, strong bees, are quite capable of piercing a corolla or calyx wall. In the tropics particularly, a number of plant species have evolved structures or mechanisms to prevent nectar theft. For example, some flowering plants have extrafloral nectaries that attract ants, which in turn patrol the regions of the flower where a nectar-robbing bee would make a slit (van der Pijl, 1954).

Nesting behavior

All carpenter bees build their nests in plant material, either in woody plant stems or in tree trunks, twigs, or structural timber (Figure 7.2). Multivoltine species may make short tunnels with one to six cells, and then may commence to gnaw away, using their mandibles, additional tunnels once the first is completed; univoltine species may build and provision up to four tunnels in a season (Gerling *et al.* 1989). After the tunnels have reached a minimum length of about 4 cm, and food plants are abundant, breeding cells are constructed within the tunnels (Anzenberger 1977). It is estimated that construction and provisioning of a cell requires one to two days during peak flowering; about five days of work are required to produce two offspring (Anzenberger 1977). Another calculation of the amount of foraging that a female *Xylocopa* needs to carry out to provision one nest was provided by Louw and Nicholson (1983). They observed *X. capitata* as it foraged almost exclusively on one plant, *Virgilia divaricata*, and calculate that a female bee needs to harvest pollen and nectar from about 1 700 unvisited flowers to rear one offspring.

Foraging duration and the quantity of pollen brought to the nest have considerable variability, linked to species differences, as well as availability, distance and quality of flowers (Gerling *et al.* 1989). The mass of the provisions of three *Xylocopa* in East Africa is given in Table 7.2. Khapil

Table 7.2

WEIGHTS OF *XYLOCOPA* PROVISIONING MASSES

SPECIES	WEIGHT OF PROVISIONING MASS
<i>Xylocopa imitator</i>	1.2-1.3 g
<i>Xylocopa flavorufa</i>	2.0-2.1 g
<i>Xylocopa nigrita</i>	2.4-2.5 g

and Dhaliwal (1969) estimated that it takes a female *X. genstrata* 20.5 flights over a period of 75 hours to provision one cell. The provision mass content has been estimated to be composed of 55.9 percent glucose, 3 percent saccharose, and the remainder insoluble remnants of pollen (for *X. violaceae*, Florentin 1904, in Anzenberger 1977).

The preparation of bee bread in brood cells proceeds from the pollen that has been collected during foraging. Studies show that a single female carpenter bee may make four to eight cells during her life (Bonelli 1976; Hurd and Moure 1960; Camillo and Garafaro 1982). The number of cells provisioned will be governed by the availability of resources. The pollen mass in large carpenter bee nests is reported to range from 2.0-2.5 g in each cell.

Once the brood cells have been completed, the female bee remains in the burrow, guarding the nest from invaders. She may make foraging trips two or three times per day, presumably feeding herself.

PESTICIDE EXPOSURE ROUTES FOR XYLOCOPA

Life cycles and exposure routes

Factors that are likely to increase exposure of *Xylocopa* to chemicals may include their sociality. This could conceivably increase the level of pesticide poisoning as a result of food sharing with other adult and young bees present in the nest.

As mentioned, *Xylocopa* males of some species, such as *X. virginica*, are often found congregating around patches with rich floral resources, likely to be visited by females (Baird 1986; Barthell and Baird 2004). Avoiding pesticide application to these foraging sites would mitigate pesticide exposure—something that should be, but rarely is, carefully considered when supplying and maintaining hedgerows and floral banks near crops.

One important aspect of *Xylocopa* natural history is the use and defense of mating territories by males. This is relevant because male carpenter bees potentially spend long periods of time exposed, while hovering next to their mating territory, in areas adjacent to agriculture. These male territorial areas can often be found at the edge of forests or of cropping systems, or other habitat gradients.

Foraging exposure routes

Whether a bee is a generalist or specialist forager on flowering plants may also determine the level of risk of exposure to chemical pesticides. This behavior may readily change due to flowering or weather conditions, and the availability of flowers during one season or in one area.



Exposure through nesting resources

Sequential generations of carpenter bees—which nest in wood—often return to the nesting sites of their mothers. This is a possible route of pesticide exposure resulting from the reuse of parental nest substrates that have been exposed to pesticides. Reuse of parental nest substrates has been found to be more common in tropical bees, which produce more than one generation per year (Roubik 1989).

FORAGING, NESTING AND PROVISIONING BEHAVIOUR OF *AMEGILLA*

There are about 250 species of *Amegilla*. They are medium-sized bees with most having metallic blue or green bands on the body. This bee genus is widely distributed and is found throughout Africa and Madagascar, Europe and the Mediterranean basin, the Canary Islands, central and southeast Asia, Japan and Australia. Members of this genus nest in the ground and sometimes form aggregations.

The majority of *Amegilla* are mainly fossorial (adapted to digging and nesting underground). *Amegilla* often nest gregariously in vertical burrows in the ground or horizontally in soil embankments; they are also found in sandstone or artificial substrates (Michener 1960). Dried river-banks and old clay houses are favoured nesting sites for *Amegilla*. In western Australia, *A. dawsoni* nests in dense aggregations in clay pans (Houston 1991). In the absence of physical disturbances to the soil, nesting sites of blue-banded *Amegilla* species can persist for many years. They may contain thousands of cells due to the bees' natural tendency to return to their natal sites (Michener 1960; Cardale 1968).

Nest provisioning behaviour has been found to vary from species to species. In *A. dawsoni*, females have a well-defined brood cell cycle involving cell construction, application of cell linings, provisioning, egg laying and cell capping (Alcock 1999). Recent studies show that *A. dawsoni* makes 6 to 26 foraging trips per day (Neff 2008) while *Amegilla cingulata* has a limited foraging range of 300 m and females make on average nine foraging flights per day. These bees are known to commonly visit blue flowers, but also forage on other flowers, such as the yellow flowers of tomato (*Solanum lycopersicum*) (Figures 7.3a and 7.3b).

In regard to crop pollination, *Amegilla* have the capacity to buzz-pollinate. This makes them effective pollinators of plants of the family Solanaceae. *Amegilla* have been identified as good pollinators of crops in greenhouses. *Amegilla chlorocyanea* is used in Australia for the pollination of tomato in greenhouses where a total of 282 female nesting bees are required per hectare (Hogendoorn 2007). Two species of *Amegilla* are documented as principal and efficient pollinators of wild cardamom in India (Kuriakose *et al.* 2009).

Figure 7.3a

AMEGILLA SP. ON *CADABA ROTUNDIFOLIA*, IN TURKANA, NORTHERN KENYA



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Figure 7.3b

TETRALONIELLA SP. VISITING A FLOWER OF *ORTHOSIPHON*, LAIKIPIA, KENYA



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PESTICIDE EXPOSURE ROUTES FOR *AMEGILLA*

Clearly any nesting aggregations of *Amegilla*, if found, need to be protected from pesticides. These bees are very active and dominant crop pollinators —estimated to pollinate, for example, 30 percent of the crops in Australia (Dollin and Bartly 2000) and probably similar proportions in many tropical countries. Thus, a focused effort to better understand their life history and avoid their active foraging periods when pesticides are applied would be warranted.

An example of the particular aspects of *Amegilla* natural history that may need special consideration is the behavior of the males. Male bees of the genus *Amegilla* sleep by clasping onto vegetation during the night (Figure 7.4); they thus may be vulnerable to pesticide sprays that take place at night, even though this time of application is often recommended as a possible mitigation measure.

Figure 7.4

SLEEPING MALE *AMEGILLA*, KENYA



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CONCLUSIONS

There is an urgent need to carry out more research on the pesticide exposure risk to large bees. Data collected should furnish more details on life history, nesting and foraging behavior, quantify the amounts and volume of nectar and pollen load collected by an individual female, and establish insecticide levels in large bees and pollen provisions in bee nests, as emphasized in other chapters in this book. Further actions should include monitoring levels of chemical accumulation for possible lethal and sub-lethal effects on foraging behavior. There is always the need to consider Integrated Pest Management (IPM) programmes that embrace pollinator and environmental protection (see Chapter 1).

Management practices that can reduce risks to *Xylocopa* and *Amegilla*

- Identify cropping systems (for example passion fruit) where these bees play a role and carefully manage pesticide application. Note that there is considerable variation in behaviour and natural history among different regions/habitats, which need to be taken into account.
- Avoid spraying known nesting sites in dry wood or field edges with natural vegetation.
- Map and protect aggregated/communal nest sites.
- Construct and protect artificial nest sites for carpenter bees. This needs to be based on studies of their nesting biology.



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*View inside a managed hive of the stingless bee *Meliponula ferruginea* at Kakamega Forest, Kenya before harvest of honey. In the foreground at the brood chambers (hidden under sheaths of involucre), the pollen stores are in the center and the honey pots are located in the far end of the hive.*

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Short biography: Agronomist Engineer, postgraduate (MS and Ph.D.) in Entomology with expertise in beekeeping management and protection (Ecotoxicology).

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Short biography: MS in Biological Science (Cellular and Molecular Biology), BA (1998), MA in Biological Sciences (Zoology) (2001) and Ph.D. in Biological Sciences (Zoology) from Universidade Estadual Paulista Júlio de Mesquita Filho (2006). Applied Zoology, mainly in toxicity, morphology, insecticides, bees, synanthropic animals, urban ants: association with bacteria, control and management, integrated pest control in hospitals.

Sheila R. Colla

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Short biography: Ph.D. in Biology (2012) York University, Toronto, Ontario, Canada. Research on the life history, taxonomy, behaviour, and conservation of North American bumblebees. Project Leader at WPC, a non-profit organization that aims to bring species back from the brink of extinction.

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Short biography: MS in Biological Sciences (Cellular and Molecular Biology), currently Ph.D. student and researcher at the Center for the Study of Social Insects IB-UNESP, Rio Claro. Student of social insects: biology, behavior, control, toxicity, bee-plant interaction, conservation of pollinators, and bee products.

Barbara Gemmill-Herren

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Short biography: Pollination work in Kenya from 1998 to 2003. Lecturer, University of Nairobi, Advanced Plant Ecology, Thesis adviser of post graduate students. Global Project Coordinator for the GEF/UNEP/FAO funded Global Pollination Project since 2004, implemented in seven countries (Brazil, Ghana, India, Kenya, Nepal, Pakistan, South Africa).

Mary W. Gikungu

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Short biography: BS in Botany and Zoology from University of Nairobi, MS in Biology of Conservation from University of Nairobi and Ph.D. in Natural Science and Mathematics from Bonn University. Bee/Pollination Ecology specialist, invertebrate taxonomy and ecology. Multidisciplinary projects on effects of habitat fragmentation and climate change on ecosystem services and food security, adviser to local and international postgraduates.

Jitendar Kumar Gupta

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Short biography: Ph.D. in Zoology (H. P. University, Shimla). Senior Scientist (Professor); teaching, research and extension activities and thesis adviser in apiculture.

Irene Koomen

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Short biography: MA in biology, Ph.D. (University of Kent, Canterbury, UK) on biological control of anthracnose in mango. Specialization in capacity development in integrated pest management and crop protection.

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Short biography: MA (1979), Ph.D. (1982), Zoology. Professor in the Department of Biology and researcher at the Center for Study of Social Insects, UNESP, Rio Claro. Emphasis on ecotoxicology and conservation of social bees, biology, behavior, control, toxicity, bee-plant interaction, conservation of pollinators and bee products.

Dino J. Martins

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Short biography: Ecologist and evolutionary biologist. BA in Anthropology from Indiana University (1999), Ph.D. in Organismic and Evolutionary Biology, Harvard University (2011). Insect-plant interactions and mutualism, biodiversity conservation, evolution and ecology of social insects (ants, bees, wasps, termites) and the biology of vectors as part of environmental change.

Roberta Cornélio Ferreira Nocelli

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Short biography: Biologist (1991), Ph.D., Molecular and Cell Biology, Universidade Estadual Paulista Júlio de Mesquita Filho (2003). Current research ecotoxicology of bees and adjunct professorship at Center for Agricultural Sciences UFSCar.

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Short biography: Ph.D., Zoology, Universidade Estadual Paulista Júlio de Mesquita Filho (2006). Works in behavior, toxicology and biology of bees.

David Ward Roubik

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Short biography: Ph.D. University of Kansas (1979). Africanized honey bee impact in tropical America. Senior Scientist, Staff Entomologist, field naturalist. Stingless bee, honey bee and orchid bee specialist, pollination ecologist of wild and cultivated plant species.



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Short biography: Formed in Biological Sciences at the Universidade Estadual Paulista “Júlio de Mesquita Filho”, with postdoctoral completed in 2011 in the same university. She has experience in toxicology, with emphasis on analysis of the effect of pesticides on bees.

Cory Sheffield

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Short biography: BS, MS, Acadia University (Nova Scotia), Ph.D. Environmental Biology, University of Guelph (Ontario). Research Scientist and Curator of Invertebrate Zoology, Royal Saskatchewan Museum, bee taxonomy, conservation, ecology, and their role as pollinators.

Erhard Strohm

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Short biography: Diplom and Ph.D., Zoology (Würzburg University), Professor, University of Regensburg, evolutionary ecology with and resource allocation, chemical ecology, insect-microbe - interactions and host-parasite interactions.

Harold van der Valk

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Short biography: MS in Biology, Wageningen University. Specialized in pesticide ecotoxicology in the tropics and (semi-)arid ecosystems, West Africa. Consultant, FAO, WHO and Wageningen University and Research Centre, pesticide management and environmental toxicology.

ANNEX 1

ASPECTS DETERMINING RISK OF PESTICIDES TO BEES:

SURVEY FORM TO ESTABLISH A RISK PROFILE

In order to elaborate a risk profile for bees of pesticide use in a specific crop, information is needed on three aspects: (i) the toxicity of the pesticide; (ii) the probability of exposure of the bee to that pesticide; and (iii) the population dynamics of the bee species in question.

Pesticide **toxicity** data have mainly been generated for the Western honey bee (*Apis mellifera*), but much less so for other *Apis* species or non-*Apis* bees (both wild and managed). Increasingly, however, toxicity tests are being done with non-*Apis mellifera* species, although not all of these have found their way into the international published literature.

The probability and degree of **exposure** to pesticides depend on cropping and pesticide application practices, pesticide properties, attractiveness of the crop to bees, and bee biology (in particular phenology and behaviour). Data on such aspects of exposure, for a given crop in a given country or region, may be available from agricultural extension services, pesticide registration authorities, bee experts, agronomists and environmental scientists.

Finally, the **population dynamics** of the bee species will determine how an observed effect of the pesticide (either lethal or sublethal) may alter long-term survival of the population.

It is unlikely that the information listed in the questionnaire is all available from one institution or person in a given country. It is certainly necessary to consult with agronomists, extension services and farmer associations working in the focal crops to obtain cropping and pesticide use data, with the pesticide registration authority and research organizations to obtain pesticide property and toxicity data, and with bee and pollination experts to obtain bee biology information. All the information has been compiled into one questionnaire, however, to underline the interdisciplinary nature of pesticide risk assessment.

Some information will be available from the published literature, other data may be obtained from local unpublished report or studies, or be provided through expert opinion. All such information can be very relevant for risk assessment and should be compiled. However, to be able to allow proper interpretation of the data, it is important to provide the source(s) of each input in the table, irrespective of whether they are published reports/articles or personal



communications. If data/information is unavailable or unknown, please also explicitly mention this, as it will help identify gaps in our knowledge. Finally, it is helpful to list all the institutions and persons that were consulted for the assessment.

A. Case identity

The assessment can be done on a country-wide basis if the cropping systems and bee complexes are similar throughout the country, or on a regional basis if important differences exist within the country.

Country:	
Region (optional):	
Crop:	Number of growing seasons per year:
Main bee species/groups visiting the crop:	Is species an important pollinator of the crop?
1.	yes/ no/ not known
2.	yes/ no/ not known
3.	yes/ no/ not known

B. Exposure – crop factors

Assessment of whether there is a possibility of exposure of bees to the pesticide in this crop.

This information should allow a first evaluation as to whether bees may be exposed to pesticides in the crop. This is the case when they are likely to be active foraging for pollen or (extrafloral) nectar in the crop, or when they are collecting nesting materials, when (or just after) pesticides are applied to that crop. Bees may also be exposed if a systemic pesticide has been applied to a previous rotational crop. If exposure is unlikely, pesticide risk to wild bees is considered to be low, and obtaining information on the aspects below is not necessary.

FACTOR		REMARKS	SOURCE OF INFORMATION (refer to section G)
Surface area under the crop		Within the overall area for which the assessment is done	
○ Overall size		ha	
○ Patchiness		Percent of total area with this crop	
Period(s) in the growing season when pesticides are applied to the crop:		Note the month(s)/ date(s)/ or timing relative to emergence, flowering or harvest	
Period(s) in the year when the crop is grown:		Note the month(s)	
Period(s) in the year when the crop flowers:		Note the month(s)	
Period(s) in the year when the bee species/groups are active foraging or collecting nesting materials outside the nest/hive:	1.	Note the species/group and the month(s)	
	2.		
	3.		
Are any weeds flowering in the crop that may be attractive to bees? If yes: Period(s) during the crop season when weeds are flowering:		yes/no if yes: note the month(s)	
Does the crop have extrafloral nectaries that may be attractive to bees?		yes/no	
Is the crop regularly infested with honeydew producing insects (e.g. aphids, scale insects) that may be attractive to bees?		yes/no	
Do the bees likely visit the treated crop to collect water (e.g. dew on crop? open water in/near crop?)		yes/no	
Are any systemic pesticides applied as soil treatment or seed treatment to a previous rotational crop?		yes/no	
Do male bees “roost” in the crop, at night?		yes/no	
Do male bees establish mating sites in the crop?		yes/no	



C. Exposure – bee biology factors

This section contains relevant information on bee biology that may partly determine pesticide risk. Please provide information for each bee species/group identified under section A. Please also provide references to published literature or unpublished research reports when possible. Indicate when information is expert opinion, and note the name(s) of the expert(s). If the information is unavailable, please explicitly note this.

FACTOR	BEE SPECIES/GROUP			REMARKS	SOURCE OF INFORMATION (refer to section G)
	1:	2:	3:		
Period of the day when foraging or collecting nesting materials (outside the nest):					
Time spent foraging, or collecting nesting material, per day ("time-out-of-nest/hive"):				hours	
Number of days spent foraging on the crop (for an individual bee):				days	
Number of days spent foraging on the crop (for the colony):				days	
Number of different nectar and pollen plant species used during crop flowering:					
Quantity of pollen collected per day:				mg per bee per day	
Quantity of nectar collected per day:				mg per bee per day	
Quantity of nectar consumed per day:				mg per bee per day	
Body weight:				mg	
Percent of pollen self-consumed:					
Percent of pollen fed to brood:					
Percent of nectar self-consumed:					
Percent of nectar fed to brood:					
Location of nest in relation to crop field –					
Inside/outside crop field:					
Approximate distance from crop field:				m	
Bee foraging range –					
Average distance from nest:				m	
Maximum distance from nest:				m	
Collective pollen and/or honey storage in the nest (social bees):				yes/no	
Other aspects of bee biology or behaviour that may impact exposure:					



E. Impact and recovery – pesticide properties

This section contains relevant information on the properties of all the pesticide active ingredients used on the crop. These aspects are independent of the actual pesticide use practices described above. Provide references to published literature or unpublished research reports when possible. If the information is unavailable, explicitly note this as well. Use more pages, if needed.

PESTICIDE PROPERTY	BEE SPECIES/GROUP			REMARKS	SOURCE OF INFORMATION (refer to section G)
	1:	2:	3:		
Pesticide i.					
Contact LD ₅₀ (adult)				□g/bee	
Oral LD ₅₀ (adult)				□g/bee	
Brood toxicity				only for IGRs	
Foliar residual toxicity				in days; note application rate	
Pesticide ii.					
Contact LD ₅₀ (adult)				□g/bee	
Oral LD ₅₀ (adult)				□g/bee	
Brood toxicity				only for IGRs	
Foliar residual toxicity				in days; note application rate	
Pesticide iii.					
Contact LD ₅₀ (adult)				□g/bee	
Oral LD ₅₀ (adult)				□g/bee	
Brood toxicity				only for IGRs	
Foliar residual toxicity				in days; note application rate	
Pesticide iv.					
Contact LD ₅₀ (adult)				□g/bee	
Oral LD ₅₀ (adult)				□g/bee	
Brood toxicity				only for IGRs	
Foliar residual toxicity				in days; note application rate	
Pesticide v.					
Contact LD ₅₀ (adult)				□g/bee	
Oral LD ₅₀ (adult)				□g/bee	
Brood toxicity				only for IGRs	
Foliar residual toxicity				in days; note application rate	

Etc.

F. Impact and recovery – life history and population dynamics factors

This section contains relevant information on bee life histories and population dynamics that may partly determine pesticide risk. Please provide information for each bee species/group identified under section A. Please also provide references to published literature or unpublished research reports when possible. Indicate when information is expert opinion, and note the name(s) of the expert(s). If the information is unavailable, please explicitly note this.

FACTOR	BEE SPECIES/GROUP			REMARKS	SOURCE OF INFORMATION (refer to section G)
	1:	2:	3:		
Individual metabolic rate					
Degree of sociality					
Fraction of population/colony active out of the nest/hive (social bees)					
Time to reproductive age of queen/reproductive female (egg-adult)				days	
Number of offspring per queen/reproductive female					
Number of generations per year					
Population growth rate [note: as product of previous 3 factors]				Colony multiplication factor per unit time; or number per reproductive female per unit time	
Number of swarms per colony or reproductive events per year					
Migration and dispersal distance				km	



G. Sources

In this section, all the institutions and persons consulted are listed, even if they were not able to provide information or data.

REFERENCE IN PREVIOUS SECTIONS (NO.)	INSTITUTION OR PERSON CONSULTED	ASPECT	CONTACT DETAILS (e-mail address and/or telephone number)

Etc.

References to reports, articles, studies, etc. can be listed here.

REFERENCE IN PREVIOUS SECTIONS (NO.)	TITLE OF REPORT, ARTICLE, STUDY	AUTHOR(S)	PUBLICATION DETAILS

Etc.

ANNEX 2¹

PESTICIDES REGISTERED ON THE FOCAL CROPS – BRAZIL

ACTIVE INGREDIENT	TYPE	SYSTEMIC	IGR	LD ₅₀ HONEY BEE (µg/bee)		LD ₅₀ BOMBUS SPP. (µg/bee)	FOLIAR RESIDUAL TOXICITY (hours or days)	REGISTERED ON	
				LOWEST	ORAL			MELON	TOMATO
Abamectin	I, A	Lim.	No	0.002			8-72hr	X	X
Acephate	I, A	No	No	0.36		3.69 (<i>B. terrestris</i>)	>72hr	X	X
Acetamiprid	I	Yes	No	8.1	14.5	2.1 (<i>B. patagiatus</i>)		X	X
Alanycarb	I	No	No	0.80					X
Alpha-cypermethrin	I	No	No	0.036		0.15 (<i>B. terrestris</i>)			X
Anilazine	F	No	-	100					X
Azocyclotin	A	No	No	>5					X
Azoxystrobin	F	Yes	-	>25				X	X
Bacillus thuringiensis	I	No	No	>0.1				X	X
Benalaxyl	F	Yes	-	>100					X
Benfuracarb	I	Yes	No	0.29					X
Benzalkonium chloride	F, B	?	-	n.a.					X
Beta-cyfluthrin	I	No	No	0.001				X	X
Beta-cypermethrin	I	No	No	0.13					X
Bifenthrin	I, A	No	No	0.013			>24hr	X	X
Bitertanol	F	No	-	104				X	
Boscalid	F	Lim.	-	100				X	X
Bromuconazole	F	Yes	-	100					X
Buprofezin	I, A	No	Yes	>200				X	X
Captan	F	No	-	26.4				X	X
Carbaryl	I, PGR	Lim.	No	1.70		3.84 (<i>n.i.</i>)	2-14d		X
Carbofuran	I, N	Yes	No	0.15			>5d		X
Carbosulfan	I	Yes	No	0.68			3.5d		X

follows on the next page →

¹ **Registered pesticides:** AgroFit database, Ministério da Agricultura, Pecuária e Abastecimento (2011) [30]; **Type, systemicity, IGR:** Tomlin (2011) [37], Footprint PPDB (2011) [34]; **Acute LD₅₀ honey bee** (oral or contact): FAO/OSU (2011) [33]. If missing in previous, Footprint PPDB (2011) [34] and Footprint BPDB (2011) [35] – *in italics in table*; **Acute LD₅₀ bumblebee:** Mommaerts & Smagghe (2011) [36]; **Foliar residual toxicity:** Pacific Northwest Extension [88] & Florida Cooperative Extension Service [87]; determined for the honey bee at maximum normal US application rates.



ACTIVE INGREDIENT	TYPE	SYSTEMIC	IGR	LD ₅₀ HONEY BEE (µg/bee)		LD ₅₀ BOM-BUS SPP. (µg/bee)	FOLIAR RESIDUAL TOXICITY (hours or days)	REGISTERED ON	
				LOWEST	ORAL	LOWEST		MELON	TOMATO
Cartap hydrochloride	I	Yes	No	10				X	X
Chlorfenapyr	I, A	Lim.	No	0.12			<4h	X	X
Chlorfluazuron	I	No	Yes	>100					X
Chromafenozide	I	No	Yes	>100					X
Chlorothalonil	F	No	-	181				X	X
Clethodim	H	Yes	--	>100					X
Clothianidin	I	Yes	No	0.044	9.92			X	X
Copper hydroxide	F	No	-	>100				X	X
Copper oxychloride	F	No	-	15				X	X
Copper oxyde	F	No	-	>116					X
Copper sulfate	F	No	-	>11				X	
Cyazofamid	F	No	-	>100					X
Cyfluthrin	I	No	No	0.019		0.13 (n.i.)	>24h		X
Cymoxanil	F	Yes	-	25	100				X
Cypermethrin	I	No	No	0.03			>3d		X
Cyproconazole	F	Yes	-	100	1000			X	
Cyprodinil	F	Yes	-	316					X
Cyromazine	I	Yes	Yes	20			<2h	X	X
Deltamethrin	I	No	No	0.017		0.6 (B. terrestris)	<4h	X	X
Diafenthiuron	I	No	No	1.5				X	X
Difenoconazole	F	Yes	-	101	187			X	X
Diiflubenzuron	I	No	Yes	100					X
Dimethoate	I, A	Yes	No	0.098		4.8 (B. terrestris)	3d		X
Dimethomorph	F	Yes	-	100					X
Dodec-7-enyl acetate	Ph	No	-	n.a.					X
Esfenvalerate	I	No	No	0.045			24h		X
Ethion	I, A	No	No	4.18				X	X
Etofenprox	I	No	No	0.13					X
Etoxazole	A	No	Yes	200					X
Famoxadone	F	No	-	>63					X
Fenamidone	F	Yes	-	75	160			X	X
Fenamiphos	N	Yes	No	1.43				X	X
Fenarimol	F	Yes	-	100				X	
Fenpropathrin	I, A	No	No	0.05			24h		X
Fenpyroximate	A	No	Lim.	15.8					X
Fenthion	I	No	No	0.056				X	

follows on the next page →

ACTIVE INGREDIENT	TYPE	SYSTEMIC	IGR	LD ₅₀ HONEY BEE (µg/bee)		LD ₅₀ BOMBUS SPP. (µg/bee)	FOLIAR RESIDUAL TOXICITY (hours or days)	REGISTERED ON	
				LOWEST	ORAL	LOWEST		MELON	TOMATO
Flazasulfuron	H	Yes	-	>100					X
Fluazifop-P-butyl	H	Yes	-	112	200				X
Fluazinam	F	No	-	100					X
Fluquinconazole	F	Yes	-	>100				X	
Flutriafol	F	Yes	-	5				X	
Folpet	F	No	-	33.8				X	
Formetanate	I, A	No	No	10.6					X
Gamma-cyhalothrin	I	No	No	0.005					X
Hexadec-11-enyl acetate	Ph	No	-	n.a.					X
Hexadeca-E-11	Ph	No	-	n.a.					X
Imibenconazole	F	Yes	-	125				X	
Imidacloprid	I	Yes	No	0.004		0.02 (<i>B. terrestris</i>)	>24h	X	X
Indoxacarb	I	No	No	0.40				X	X
Iprodione	F	No	-	400				X	X
Iprovalicarb	F	Yes	-	>199				X	X
Kasugamycin	F, B	Yes	-	>25					X
Kresoxim-methyl	F	No	-	14				X	X
Lambda-cyhalothrin	I	No	No	0.093		0.11 (<i>n.i.</i>)	>24h		X
Lufenuron	I, A	No	Yes	197					X
Malathion	I	No	No	0.47			5.5d		X
Mancozeb	F	No	-	>20				X	X
Maneb	F	No	-	12					X
Metalaxyl-M	F	Yes	-	200				X	X
Metam sodium	F, N, H, I	No	No	36.2					X
Methamidophos	I, A	Yes	No	0.1			24hr		X
Metconazole	F	Yes	-	97				X	X
1-methylcyclopropene	PRG	No	-	n.a.				X	X
Methiocarb	I, A, M	No	No	0.37			>3d		X
Metiram	F	No	-	40				X	X
Methomyl	I, A	Yes	No	0.42		0.57 (<i>B. terrestris</i>)	1.5d		X
Methyl bromide	I, A, N	No	No	n.a.				X	
Methyl-eugenol	Ph	No	-	n.a.					X
Methoxyfenozide	I	No	Yes	>100					X
Metribuzin	H	Yes	-	35					X
Mevinphos	I, A	Yes	No	0.086			<1.5d	X	X

follows on the next page →



ACTIVE INGREDIENT	TYPE	SYSTEMIC	IGR	LD ₅₀ HONEY BEE (µg/bee)		LD ₅₀ BOM-BUS SPP. (µg/bee)	FOLIAR RESIDUAL TOXICITY (hours or days)	REGISTERED ON	
				LOWEST	ORAL	LOWEST		MELON	TOMATO
Milbemectin	A	Lim.	No	0.025	0.46				X
Myclobutanil	F	Yes	-	>7				X	
Napropamide	H	Yes	-	121					X
Novaluron	I	No	Yes	>100					X
Oxytetracycline	B	Yes	-	>100					X
Permethrin	I	No	No	0.029		0.81 (<i>B. terrestris</i>)	0.5-2d		X
Phenthoate	I, A	No	No	0.3					X
Phorate	I, A, N	Yes	No	1.12		1-2 (<i>B. lucorum</i>)	24h		X
Pirimicarb	I	Yes	No	6.21		8.5 (<i>B. terrestris</i>)	<2h		X
Prochloraz	F	No	-	37.4					X
Procymidone	F	Yes	-	100				X	X
Profenofos	I, A	No	No	1.23					X
Propargite	A	No	No	15					X
Propamocarb hydrochloride	F	Yes	-	100	116				X
Propiconazole	F	Yes	-	14.1					X
Propineb	F	No	-	200					X
Prothiofos	I	No	No	n.a.					X
Pymetrozine	I	?	No	117			<2h	X	X
Pyraclostrobin	F	Lim.	-	73				X	X
Pyrazophos	F	Yes	-	0.65	0.84			X	
Pyridaphenthion	I	No	No	0.08					X
Pyrimethanil	F	Lim.	No	>100				X	X
Pyriproxyfen	I	No	Yes	>100				X	X
Quinomethionate	A, F	No	No	n.a.				X	
Quintozene	F	No	-	100					X
Quizalofop-P-ethyl	H	No	-	71					X
Spinosad	I	No	No	0.003			<2h		X
Spirodiclofen	I, A	No	Yes	>196					X
Spiromesifen	I, A	No	Yes	>200				X	X
Streptomycin	B	Yes	-	>100					X
Sulphur	F, A	No	-	1051				X	X
Tebuconazole	F	Yes	-	176				X	X
Tebufozide	I	No	Yes	234			<8h		X
Teflubenzuron	I	No	Yes	1000					X
Tetraconazole	F	Yes	-	>130				X	X
Tetradec-3,8,11-enyl acetate	Ph	No	-	n.a.					X

follows on the next page →

ACTIVE INGREDIENT	TYPE	SYSTEMIC	IGR	LD ₅₀ HONEY BEE (µg/bee)		LD ₅₀ BOMBUS SPP. (µg/bee)	FOLIAR RESIDUAL TOXICITY (hours or days)	REGISTERED ON	
				LOWEST	ORAL	LOWEST		MELON	TOMATO
Tetradec-3,8-enyl acetate	Ph	No	-	n.a.					X
Tetradec-9-enyl acetate	Ph	No	-	n.a.					X
Tetradifon	A	No	No	60.4					X
Thiabendazole	F	Yes	-	>10				X	
Thiacloprid	I	Lim.	No	17.3				X	X
Thiamethoxam	I	Yes	No	0.005			7-14d	X	X
Thiophanate-methyl	F	Yes	-	>70				X	X
Triadimefon	F	Yes	-	25				X	
Triazophos	I, A, N	No	No	0.06					X
Trichlorfon	I	No	No	0.4			3-6h	X	X
Triflumizole	F	Yes	-	56.6				X	
Triflumuron	I	No	Yes	>100					X
Trifluralin	H	No	-	62.3					X
Triforine	F	Yes	-	>10				X	
Zeta-cypermethrin	I	No	No	0.002			>1d		X
Zoxamide	F	No	-	>153					X
(Z,Z,Z)-3,6,9-tricosatriene	Ph	No	No	n.a.					X

n.a = data not available; ? = possibly; n.i. = species not identified; - = no insecticide and therefore not applicable; Lim. = limited; d = day; h = hour; min = minute; mg = milligram; mL = millilitre; µL = microlitre
A=acaricide, I=insecticide, F=fungicide, H=herbicide, N=nematicide, PGR=plant growth regulator, Ph=pheromone, M=molluscicide, B=bactericide, R=rodenticide

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A key element of any organism's pesticide risk is its natural history, and the routes by which it may be exposed to pesticides in foraging and nesting activities. In this respect, a series of presentations on the natural history of wild bee groups and pesticide exposure were solicited for a session on "Exploring pesticide effects on non-*Apis* bees" at the X International Symposium on Pollination, convened by the International Commission on Plant-Bee Relations, in Mexico, 27-30 June, 2011. The presentations have been more fully developed for the present publication, as a contribution to knowledge management of pollination services in sustainable agriculture. A careful look at pollinators, as presented in these chapters, can help to understand how they may live and carry out their vital functions in agroecosystems, and how farmers and land managers may - through this understanding - mitigate their impacts on key pollinator groups.



GLOBAL ACTION ON **POLLINATION SERVICES**
FOR **SUSTAINABLE AGRICULTURE**

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